Spectrochemical Studies on the Anomalous Color Reactions of Some Iron(II) Chelate Compounds

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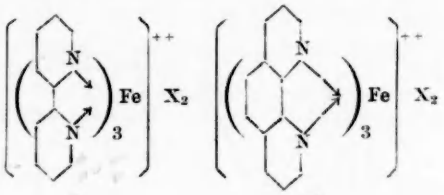
(Received August 20, 1951)

Introduction

The colors of simple salts containing ferrous ion are generally light green and the absorption spectra of their aqueous solutions have a weak, split band in the infra-red region at *ca.* 1,000 mμ and also a weak, continuous absorption in the ultraviolet region.(1) It was found by Yamasaki and his collaborators,(2) however, that the ferrous complex salts containing 2, 2′-dipyridyl and 1, 10-phenanthroline ((I) and (II))



I II

show very strong absorption bands in the visible and near ultraviolet regions which differ substantially from the absorption bands due to the ferrous ion or to the organic ligand molecules of these complexes. The position of visible bands of dipyridyl- and phenanthroline ferrous complexes are 520, 510 mμ respectively (*cf.* Fig. 1). Complex salts of other metals, such as Cu, Zn, Co and Ni, containing these ligand molecules have no such bands; therefore, the appearance of these new, strong bands seems to be characteristic of the ferrous complexes themselves.(3)

The present study deals with the absorption spectra of the peculiar color reactions shown by ferrous ions with a number of organic substances. The following organic compounds are

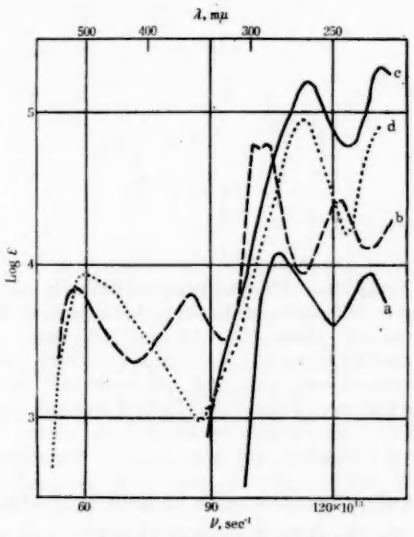


Fig. 1.—(a), 2, 2′-dipyridyl in H_2O; (b), $[Fe(dip)_3]Cl_2$ in H_2O; (c), 1, 10-phenanthroline in H_2O; (d), $[Fe(phen)_3]Cl_2$ in H_2O.

already known to show anomalous color reactions with a ferrous ion(4):

(A) 2, 2′-Dipyridyl, 1, 10-phenanthroline and their derivatives: red.

(B) Dimethylglyoxime and dioximes with similar structures: red.

(C) Compounds containing the group —C(:NOH)—CO—: blue or green.

(D) α-Carboxylic acids of pyridine, quinoline and their derivatives: brown, violet, etc.

(E) 8-Hydroxyquinoline: red.(5)

(F) Pyrocatechol and related polyphenols: violet.(6)

Of these six groups, (A) was already studied in our laboratory; also (D) was studied earlier by Ley and his collaborators(7) who showed

(1) Th. Dreisch, *Z. Phys.*, **40**, 714 (1927); Th. Dreisch and O. Kallscheuer, *Z. phys. Chem*, **B39**, 19 (1939); R. H. Potteril, O. J. Walker and J. Weiss, *Proc. Roy. Soc.*, **A156**, 561 (1936).

(2) K. Yamasaki, This Bulletin, **15**, 461 (1940); K. Yamasaki, H. Yokoi and K. Sone, *J. Chem. Soc. Japan*, **69**, 137 (1948); *Chem. Abst.*, **44**, 9295 (1950).

(3) It may be noted that the ferric complexes of these compounds also show similar bands[2], and that W. W. Brandt and G. F. Smith (*Anal. Chem.*, **21**, 1313 (1949)) reported that the dipyridyl complex of ruthenium also shows an analogous band.

(4) F. Feigl, "Chemistry of the Specific, Selective and Sensitive Reactions," 1949.

(5) R. Berg, "Die analytische Verwendung von o-Oxychinolin "Oxin" und seiner Derivate," 1938.

(6) Snell, "Colorimetric Methods of Analysis", Vol. II, 1937.

(7) H. Ley and C. Schwarte, O. Münnich, *Ber.*, **57B**, 349 (1924).

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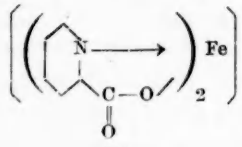
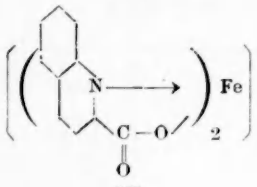
that the absorption spectra of such color reactions present great similarities with those of (A). Also in this case, a strong visible band appeared by chelate formation (III) (IV) which



III

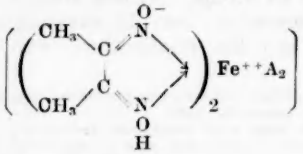


IV

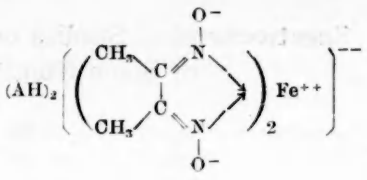
is completely different from the ligand absorption. Therefore, compounds belonging to the remaining three (B), (C) and (E), such as dimethylglyoxime, 8-hydroxyquinoline, α-nitroso-β-naphthol and nitroso-R salt which are the most common analytical reagents, were studied spectrophotometrically in regard to their anomalous coloration with a ferrous ion.

Color Reaction with Dimethylglyoxime

Tschugaeff[4] discovered that a ferrous ion shows strong red coloration in the solutions containing dimethylglyoxime or dioximes having similar structures, and bases containing nitrogen such as NH_3, amines or pyridine. This coloration is, however, rather unstable, and readily fades away in contact with air, owing to the rapid oxidation of the ferrous to ferric state which is ineffective for color production; therefore small amounts of reducing agents must be added to the solution to obtain stable coloration. As the cause of the color, Tschugaeff assumed the formation of hexacoördinated complexes such as $[Fe(DH)_2A_2]$ (DH_2= dimethylglyoxime, A=NH_3 or amines) (V), although Cambi[8] prepared pyridine-containing complex salts of somewhat different composition, and Feigl[4] considered the possibility of ionic formula, such as $(AH)_2[FeD_2]^{-2}$ (VI).



V



VI

By using hydrazine which is a reducing agent as the basic component, or by adding small amounts of sodium hydrosulfite crystals to the sample solutions, the present author has succeeded in preparing colored solutions containing a large exess of dimethylglyoxime and bases which were stable for about half an hour at least, and measured their absorption spectra in visible and near ultraviolet regions (Fig. 2). Dimethylglyoxime itself shows only

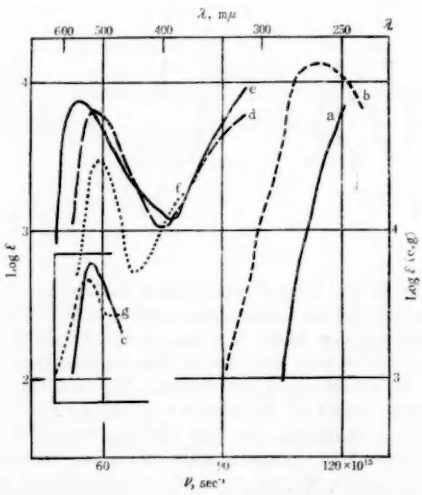


Fig. 2.—(a)~(b), dimethylglyoxime, (a) in alcohol and (b) in 0.05 *N* KOH; (c)~(f) Fe^{++} (0.00025 *M*) + DH_2 (0.0025 *M*), (c) +NH_3 (0.015 *M*), (d) +N_2H_4 (0.05 *M*), (e) +en (0.015 *M*) and (f) +pyridine (0.015 *M*); (g), Ni$(DH)_2$ colloidal solution (Juza).

a continuous absorption in the ultraviolet region which shifts in alkaline solutions to longer wave-lengths, a broad band appearing at *ca.* 260 mμ. However, the colored solution containing a ferrous ion and base shows a very strong, completely new absorption band in the visible region at *ca.* 510~550 mμ, which is quite similar to those bands of the dipyridyl, phenanthroline and α-pyridinecarboxylic acid complexes already mentioned above, and such close similarity makes it highly probable that

(8) Cambi, *Gazz. chim. ital.*, **63**, 767 (1393).

all of such bands are of similar origin in their electronic systems.

As is well known, the color of the nickel dimethylglyoxime complex is red unlike many nickel complex salts. Absorption spectra of this complex have never been reported, probably owing to its small solubility in ordinary solvents; however, the absorption curve of its colloidal solution reported by Juza[9] shows a band at *ca.* 530 mμ, which is analogous to that of ferrous complexes. This phenomenon may be connected with the fact that both the nickel atom in planar configuration and the ferrous iron atom in octahedral configuration postulated by Tschugaeff contain the same number of electrons, and they are both diamagnetic,[8] suggesting that their electronic states are much alike. Dimethylglyoxime complexes of other metals, such as Co^{III} and Cu, were known to show no similar bands.[10]

In addition to these spectral measurements, the author tried to test the validity of Tschugaeff's formula (V) or other possible structures for these complexes. The combining ratio Fe:DH determined by Job's method[11] was 1:2 (Fig. 3). However, additions of nitrogen-bearing bases are necessery for coloration, and

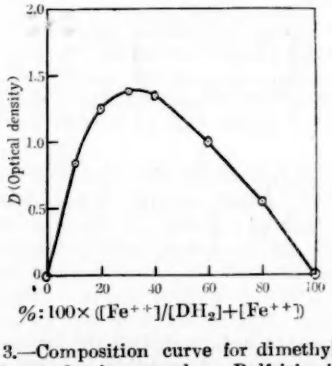
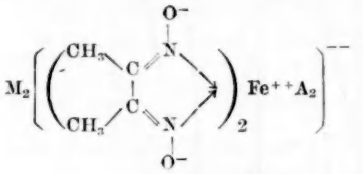


Fig. 3.—Composition curve for dimethylglyoxime-hydrazine complex; Pulfrich photometer, filter S47, d=1 cm., $[DH_2]+[Fe^{++}]$ = 0.0025 M in 0.05 M N_2H_4.

the position of a visible band depends definitely on the nature of the base. These facts suggest the coördination of the base to the central iron atom; as the coördination number of ferrous iron is usually six, the number of base molecules coördinated to an iron atom can be supposed to be two. Therefore, Tschugaeff's formula (V) seems to be in accord with these spectrochemical observations; however, the recent observation of Mathews and Diehl[12] that the analogous complex with 1,2-cyclohexanedionedioxime has a negative charge in its solution is incompatible with this structure and it supports Feigl's formula. The author's opinion is that the complex has some intermediate structure, such as (VII),

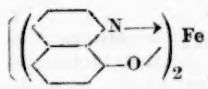


VII (M = H or cations)

which can explain all of these experimental evidences. The recent data of Mellon and Griffing[13] on the spectra of these complexes are in general agreement with ours.

Color Reaction with 8-Hydroxyquinoline

Most of the complex salts formed by 8-hydroxyquinoline with bivalent metals are yellow, orange or yellowish green in color, showing strong absorption bands in the region of 370~430 mμ with molecular extinction coefficients of several thousands; however, bands of comparable strengths are only rarely seen in the visible region.[14] From the fact that the ferrous complex is red in color,[5] it can be expected that some sort of special absorption band appears in the visible region also in this case. The author found that the ferrous complex precipitated from a weakly alkaline solution is reddish violet in color; however, this complex is very easily oxidized in air, like the dimethylglyoxime complexes, and changes into dirty black when brought in contact with air. It remains unchanged only in the mother solution containing strong reducing agents, *e. g.* hydrosulfite. Therefore the confirmation of its structure is extremely difficult, although it seems to be very probable that it has the structure (VIII) analogous



VIII

(9) Juza, R. Langheim, "Physikalische Methoden im Chemischen Laboratotium", 1937, p. 250; *Angew. Chem.* **50**, 255 (1937).

(10) H. S. French and H. E. Acly, *J. Amer. Chem. Soc.*, **48**, 847 (1927) (Cu complex); R. Tsuchida *et al.*, This Bulletin, **12**, 83, 350 (1937) (Co complexes).

(11) P. Job, *Ann. chim.*, (10) **9**, 113 (1928).

(12) J. Mathews and Jr., H. Diehl, *Iowa State Coll. J. Sci.*, **23**, 279 (1949).

(13) M. Griffing and M. G. Mellon, *Anal. Chem.*, **19**, 1017 (1947).

(14) K. Sone, unpublished results.

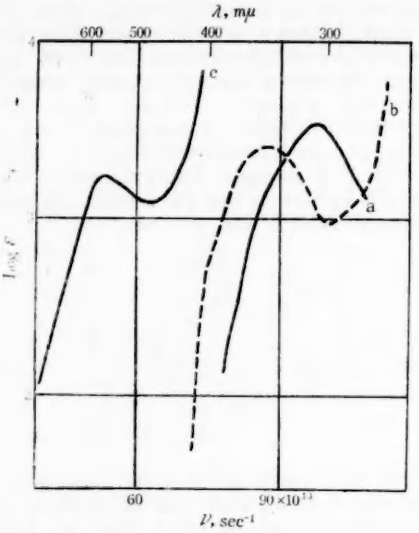
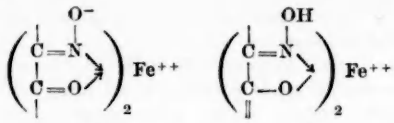


Fig. 4.—(a)~(b), 8-hydroxyquinoline, (a) in alcohol and (b) in 0.2 *N* KOH; (c), Fe^{++} (0.0003 *M*) +8-hydroxyquinoline (0.005 *M*) + KOH (0.2 *N*).

to the other metallic 8-hydroxyquinolinates precipitated under the same condition. The measurement of its absorption spectra is also difficult, and Fig. 4 (c) is that of a metastable solution of this complex produced by mixing dilute solutions of ferrous sulfate and 8-hydroxyquinoline with a large exess of the latter, which is stable for several hours in the presence of a small amount of hydrosulfite. Compared with the absorption curves of 8-hydroxyquinoline in neutral and alkaline solutions and the curves of metallic 8-hydroxyquinolinates mentioned above, it is clear that a new kind of absorption band appears at 560 mμ by the formation of this ferrous complex, the strong end absorption of its curve being due to the 8-hydroxyquinoline molecules present in excess.

Color Reaction with α-Nitroso-β-naphthol and Nitroso-R Salt

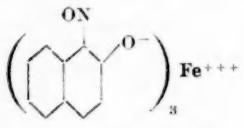
Organic compounds containing the group —C(NOH)—CO—, or the tautomeric group —C(NO):C(OH)— are known to produce blue coloration with the alkaline solutions containing ferrous ion, and complexes such as (IXa) or (IXb) are supposed to be responsible for these colors.



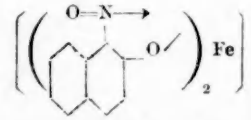
IXa IXb

Preliminary observations on isonitrosoacetone and isonitrosoacetacetic ester, the simplest compounds containing this group, revealed that the ferrous complexes of these substances possess a new strong absorption band at *ca.* 600 mμ, which is also quite different from the ligand absorption.

The same kind of reactions are observed also by aromatic compounds, and *o*-nitrosophenol and α-nitroso-β-naphthol are known to give green complexes. The latter substance and its sulfonate derivatives were studied earlier by Hoffman and Ilinsky and more recently by Sarver, Griffith and Ségal[15]. Although Hoffman considered the origin of color to be a ferric salt of the type (Xa), other authors preferred the formula (Xb) which, according to their experimental evidences, contains ferrous ion.



Xa



Xb

The present author studied the ferrous complex of α-nitroso-β-naphthol in ethyl acetate solution[16], and found that it has a strong visible band which is again quite different from the ligand absorption. The curve Fig. 5 (a) and (b) shows respectively the absorption spectra of α-nitroso-β-naphthol in alcoholic and alkaline solutions. In addition to the strong band at 370~385 mμ, a weak band appears in the latter curve at *ca.* 570 mμ. However, when the green precipitate formed

(15) O. Hoffmann, *Ber.*, **24**, 3741 (1891); M. Ilinsky and G. v. Knorre, *ibid.*, **18**, 2728 (1885); Sarvar, *Ind. Eng. Chem., Anal. Ed.*, **10**, 378 (1938); M. Griffing, M. G. Mellon, *Anal. Chem.* **19**, 1014 (1947); J. Ségal *Compt. rend.*, **227**, 1266 (1948).

(16) *Cf.* R. Vanossi *Anales soc. cient. argentina*, **131**, 226 (1941).

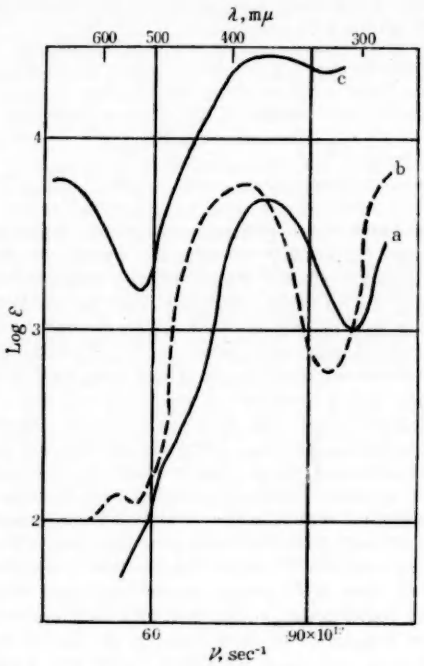
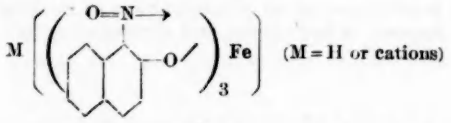


Fig. 5.—(a)~(b), α-nitroso-β-naphthol, (a) in alcohol and (b) in 0.2 *N* KOH; (c), ethyl acetate extract of ferrous complex, containing 0.000055 *M* of Fe^{++}, the initial mixing ratio of Fe^{++} to reagent being 1:6.

by the action of excess amounts of α-nitroso-β-naphthol with ferrous sulfate in weakly alkaline solution was extracted by ethyl acetate and the absorption spectra of the extracted solution was measured, a very strong band was found to appear in the neighborhood of 700 mμ, and although this complex is gradually oxidized in air changing into a black substance, It was found by rapid measurements that this new band attains its maximum absorption when about 3 moles of α-nitroso-β-naphthol are mixed with one mole of ferrous ion. Therefore the structure of this substance seems to be of hexacoördinated type, such as (Xc), instead of the conventinal formula (Xb) used by previous investigators.



Xc

Nitroso-R salt, or the sodium salt of α-nitroso-β-naphthol-3, 6-disulfonic acid, produces green soluble complex which is very stable even after standing for many hours. The characteristics of the absorption spectra of this complex are quite similar to those of α-nitroso-β-naphthol, the absorption of the new band becoming still stronger. Owing to the higher stability of the colored solution, the

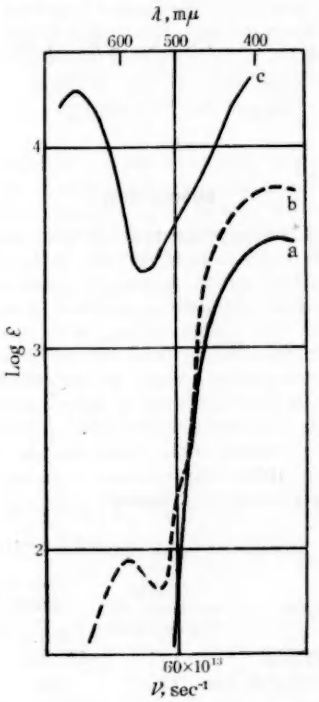


Fig. 6.—(a)~(b), nitroso-R salt, (a) in H_2O and (b) in 0.2 *N* KOH; (c), Fe^{++} (0.0001 *M*) +nitroso-R salt (0.0003 *M*) +NaOAc (0.05 *M*).

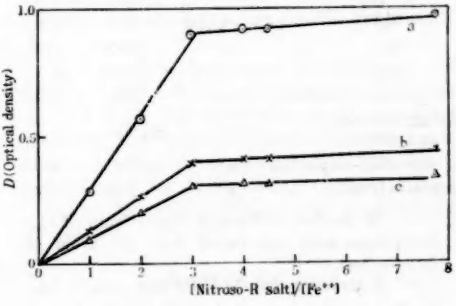
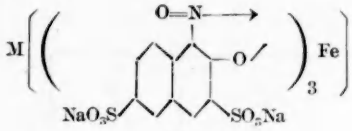


Fig. 7.—"Molar ratio" curve for nitroso-R salt complex; Pulfrich photometer, filter (a) S61, (b) S57, (c) S53, *d* = 1 cm., Fe^{++} (0.00011 *M*) +nitroso-R salt (variable amt.) in 0.05 *M* NaOAc.

exact determination of the composition of the colored complex becomes possible in this case, with the result that the molar ratio of ligand to iron is 3:1, as in the case of α-nitroso-β-naphthol, showing the validity of analogous hexacoördinated formula (XI) in this complex (Figs. 6 and 7). Recent data of Griffith *et al.* agree fairly well with the author's.



XI

Discussion

From the experimental results described above, it seems to be fairly clear that, although the structure of these complex salts are different, all of them show a strong absorption band in the visible region, with maximum wave-lengths ranging from 500 to 720 mμ and log ε of 3~4, which cannot be attributed to the ferrous ion or to the organic ligand molecules. They are only rarely observed in the complexes of other metals with these ligands and are therefore quite characteristic of these ferrous complexes studied (*cf.* Table 1).

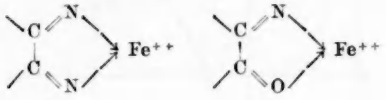
Table 1

Reagent	Probable structure of the complex	Absorption, maximum mμ	Absorption, maximum log ε*
2,2'-dipyridyl	(I)	520, 352	3.8
1,10-phenanthroline	(II)	510	3.9
α-pyridine carboxylic acid and derivs.	(III), (IV)	500~560	2.5~3**
dimethylglyoxime			
+NH_3		525	3.8
+N_2H_4	(V), (VI) or	520	3.8
+en	(VII)	550	3.9
+pyridine		510	3.5
8-hydroxyquinoline	(VIII)	560	3.2
isonitrosoacetone and derivs.	(IX)	ca. 600	
α-niroso-β-naphthol	(Xc)	ca. 700	3.8
nitroso-R salt	(XI)	720	4.3

* Molecular extinction coefficients of the complexes were calculated for one mole of ferrous ion.

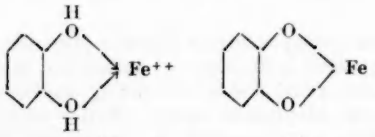
** Estimated values from the data of Ley *et al.*

From the examination of the probable structures of these complexes it becomes evident that some sort of unsaturated five-membered ring formation, such as (a) or (b), occurs in all of them in common.



a b

Of course, the positions of double bonds in these rings may be variable owing to the resonance effects. Therefore the existence of such an intramolecular ring can be assumed as a sort of chromophore which produces this kind of new absorption band. Thereby the unsaturated state of the whole ring, that is to say, the possibility of resonance of the π-electrons over all four non-metallic atoms composing the ring, seems to be essential for the color, because ethylenediamine does not form such a colored complex and 8-aminoquinoline, which has a similar structure with 8-hydroxyquinoline, only produces yellow ferrous complex[17] which may be due to the fact that their NH_2 groups become saturated with the coördination to the iron and therefore they are impossible to participate in the resonating system. It may be added that the violet compounds produced by *o*-diphenols and analogous polyphenols (*cf.* Introduction) with ferrous ion may contain an intramolecular ring of similar structure (c-1) or (c-2).



c-1 c-2

Similar chromophore action of an intramolecular organometallic ring was formerly observed by Willis and Mellor[18] in the case of diamagnetic nickel complexes, and the ferrous complexes reported in this study might be regarded as its second example. In the case of nickel complexes a close relation was found between the magnetic properties of the complexes and the appearance of a new absorption band; however, no definite relation can be found by ferrous complexes, as the band appears in both para- and diamagnetic complexes.[8]

(17) G. J. Burrows and E. Ritchie, *J. Proc. Roy. Soc. New South Wales* **72**, 113 (1939).

(18) Willis and Mellor, *J. Amer. Chem. Soc.*, **69**, 1237 (1947).

Experimental

Reagents.—Mohr's salt of extra pure grade was used as the source of ferrous ions. All the organic reagents are analysis grade samples and, when necessary, were purified by repeated recrystallization.

Preparation of Samples.—In the preparation of sample solutions with dimethylglyoxime and 8-hydroxyquinoline, a minute amount of hydrosulfite crystalls was added to the solutions, except in the case of the hydrazine complex to prevent air oxidations. Sample solutions of α-nitroso-β-naphthol were prepared by mixing the alcoholic solution of the organic reagent and aqueous solution of Mohr's salt in a 10% solution of sodium acetate, and by rapidly extracting the formed precipitate with ethyl acetate.

Spectral Measurements.—Measurements of absorption spectra were made by a Spekker spectrophotometer combined with a medium quartz spectrograph. A Pulfrich photometer was also used in the visible region. The measurements on nitroso-R salt were made with the Beckman DU spectrophotometer which was put at our disposal in the later stage of this study.

Summary

Absorption spectra of colored complex salts formed by ferrous ion with dimethylglyoxime, 8-hydroxyquinoline, α-nitroso-β-naphthol and nitroso-R salt were studied. The origin of a strong absorption band in the visible region in all of these complexes was attributed to the formation of unsaturated intramolecular five-membered rings. Determination of the composition of the complexes was made spectrophotometrically in the case of dimethylglyoxime, α-nitroso-β-naphthol and nitroso-R salt.

The author wishes to express his thanks to Prof. K. Yamasaki for his guidance. A part of the expense of this study was defrayed from a grant for Fundamental Scientific Research.

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The Structure of Surface-Denatured Protein. I. Molecular Weight and Surface Area of Horse Serum Albumin Molecule

By Kazutomo IMAHORI

(Received June 6, 1951)

As Bull and others have shown, it is possible to obtain a very thin protein film of the thickness of the order of 10 Å. by putting a small quantity of powdered protein or protein solution on the surface of concentrated salt solution[1] or water solution of propyl alcohol.[2] The protein molecule in such a film may lose its original globular shape; it is considered that the polypeptide linkage has the extended, to become the so called β-form, This can be shown by transferring the film to the surface of a solid and taking its photograph by x-ray diffraction.[3] The transformation from α-form to β-form is characteristic for the denaturation phenomenon. Therefore, the above-mentioned protein film is sometimes called surface-denatured protein. Neurath[4] has pointed out, that the surface denaturation is the most perfect denaturation. And it is quite interesting to investigate the structure of the surface-denatured protein as well as the mechanism of the surface denaturation.

This problem has been investigated by the present author from several points of view and in this paper the molecular weight and surface area of the surface-denatured horse serum albumin will be reported. The former was determined by H. B. Bull's method, but the latter by the measurement of the compressibility of the film.

Surface Balance

The author modified the so-called Wilhelmy[5]

(1) H. B. Bull, *J. Am. Chem. Soc.*, **67**, 4 (1946).
(2) H. Theorell, *Trans. Faraday soc.*, **35**, 1413 (1939).
(3) W. T. Astbury, F. O. Bell, E. Gorter and J. van Ormondt, *Nature*, **142**, 33 (1938).
(4) H. Neurath, J. P. Greenstein, F. W. Putnam and J. O. Erickson, *Chem. Rev.*, **34**, 157 (1945).
(5) Wilhelmy, *Ann. Physik.*, **119**, 177 (1863).

type surface balance and has used it instead of the Adam's type. One of the bowls of the chemical balance (made by E. Sartorius in Germany) is taken away, and a long metal wire is hung instead. A very thin glass plate is hung on the tip of the wire. The glass plate must by kept in the chromic acid mixture; it should be washed with distilled water and then dried before use, for even the slightest stain on the plate will make the measurement quite incorrect.

The length of the wire is controlled so that about one third part of the plate may be immersed under the water surface. An extremely thin glass needle is put on the tip of the needle of the balance. A scaled microscope of 40 magnification is fixed in front of the needle and is brought to its focus.

Then, putting some balance-weight on the bowl, the balance is brought in equilibrium and the position of the glass needle on the scale of the microscope is read. When some film is put on the water surface, its surface tension decreases, and the glass plate rises a little. The needle then moves to a new equilibrium position and the deflection of the needle ΔQ_1 can be expressed as

$$\Delta Q_1 = k\Delta\gamma. \tag{1}$$

Here, $\Delta\gamma$ is the surface pressure, k is a constant indicating the sensivity of the balance, contact angle being assumed to be zero. Then the balance weight on the bowl is decreased so as to bring the needle to its original position. If the decrease in weight is expressed as ΔG, the following equation must hold.

$$g\Delta G = 2(t+b)\Delta\gamma \tag{2}$$

Here, g is the gravity constant and t the thickness and b the width of the plate respectively. And thus the surface pressure can be calculated from (2) or the following relation, which can be derived from (2).

$$\Delta\gamma = \frac{g\cdot\Delta G}{2(t+b)} \tag{3}$$

In the present experiment, $t = 2\times10^{-3}$ cm. and $b = 5.5$ cm.

But it is troublesome to bring the needle just to the original position by adjusting the weight on the bowl. In such a case, the surface pressure can be calculated as follows.

When the weight on the bowl is decreased by ΔG_1 and the needle is brought to the position which is deflected from the original position by ΔQ_2, the surface pressure can be expressed as

$$\Delta\gamma = \frac{g\cdot\Delta G_1}{2(t+b)}\left(1+\frac{\Delta Q_2}{\Delta Q_1-\Delta Q_2}\right) = \frac{g\cdot\Delta G}{2(t+b)}\times\frac{\Delta Q_1}{\Delta Q_1-\Delta Q_2} \tag{4}$$

Thus $\Delta\gamma$ can be obtained from (4) by using the data of ΔQ_1 and ΔQ_2. In Fig. 1, the surface pressure—surface area curve of the horse serum albumin is shown, which is obtained by using this apparatus. Curve 1 is at pH=6, and curve 2 is at pH=10.

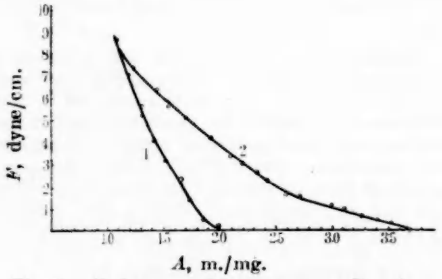

Fig. 1.—F-A curve of horse serum albumin.

Determination of Molecular Weight

The molecular weight of protein can be determined by H. B. Bull's method.[1] In Fig. 2, the film pressure F of the horse serum albumin monolayer multiplied by the film area A per milligram of protein is plotted against the surface pressure F. The experiment has been carried out under pH=6.

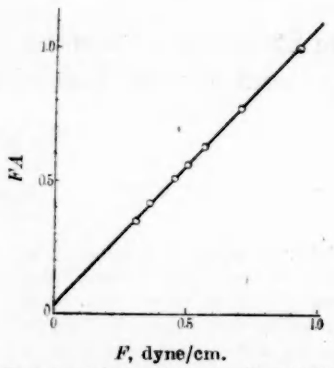

Fig. 2.—Relation between F and FA.

The protein used is the crystalline horse serum albumin which was purified by Dr. Sankichi Nakamura in Chemical Laboratory, Faculty of Science, University of Tokyo, by the sodium sulfate method. Water is distilled several times and is used as the substrate. The surface pressure is measured by the surface balance mentioned above.

Bull has pointed out that $FA-F$ curve should be given as a straight line, which is represented by the following equation:

$$FA = nRT + nS_pF. \tag{5}$$

Here, n is the mol number of the protein on the water surface and S_p is the surface area covered by one mol protein. Accordingly the extrapolated value of FA at zero surface pressure should satisfy the equation,

$$FA = nRT = (m/M)(RT), \qquad (6)$$

where R is the gas constant, T the absolute temperature and m the weight of protein put on the water surface. Thus molecular weight M can be calculated from the above equation. nS_p is equal to the gradient of the curve and can be calculated from Fig. 2. From the points on Fig. 2, values of nRT and nS_p can be calculated by using the method of least squares as follows:

$$nRT = 0.034 \pm 0.006 \text{ and } nS_p = 1.05 \pm 0.11.$$

And therefore, equation (5) can be expressed as

$$FA = 0.034 + 1.05\,F. \qquad (7)$$

And as $T = 19°C.$, the molecular weight of the surface-denatured serum albumin proves to be about $70{,}000 \pm 12{,}000$, which agrees with the data from the measurement of osmotic pressure and others.[6] This tells us that serum albumin does not split into two or more pieces, although it may lose its original shape by surface denaturation.

The surface area covered by 1 mg. of serum albumin is equal to 1.05 m.^{2} This value can be checked by the following compressibility measurement.

The Measurement of the Compressibility of the Film

As expanded film is compressed, it gradually changes into condensed film, and it becomes less and less compressible. At last, at the point where the molecules on the surface are packed most closely, the compressibility comes to its minimum. If the film is further compressed, it will be broken and the compressibility will increase again. The compressibility δ may be defined by

$$\delta = \frac{1}{A}\frac{dA}{dF}. \qquad (8)$$

Where A is the surface area per milligram of protein and F is the surface pressure. Fig. 3 shows the compressibility of the horse serum albumin at various surface areas.

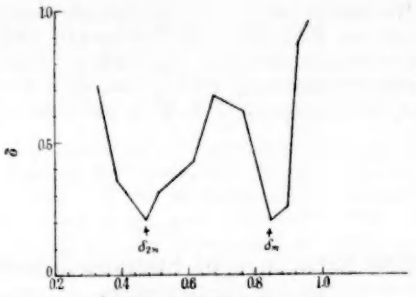

Fig. 3.—Compressibility of horse serum albumin.

As is seen from Fig. 3, the minimum compressibility δ_m has the value of 0.21. The surface pressure F_m and surface area A_m at this point have the value of 12 dyne/cm. and 0.845 m.^{2}/mg. respectively. From the value of A_m the surface area covered by one albumin molecule is calculated to be 9820 Å.^{2}

Fig. 3 shows also that the compressibility has the second minimum δ_{2m}. The surface area at this second minimum point has the value of 0.466 m.^{2}/mg. and this value is equal to about one half of that of A_m. This perhaps shows that at this point, protein has the doubled layer structure.

As is cited above, the surface area of the serum albumin molecule can be calculated from the $FA-F$ curve, and is obtained as 1.05 m.^{2}/mg. The disagreement of this value with that of A_m can be interpreted as follows.

Tonomura[7] has derived, from a statistical mechanical treatment, that if nS_p/A_m has the value of about two, the molecule on the surface is considered to have a round disk shape, while if the ratio has the value near one, it is considered to have an elongated disk shape.

From the present experiment, nS_p/A_m becomes to be equal to 1.24 and this indicates that the serum albumin molecule has an elongated disk shape.

Summary

1. The molecular weight of the surface denatured serum albumin molecule is measured from the $FA-F$ curve. The experiment has shown it to be about 70,000.

2. The surface area covered by one surface-denatured serum albumin molecule is determined to be 9820 Å.^{2} from the compressibility measurement.

(6) H. Neurath, G. R. Cooper, and J. O. Erickson, *J. Biol. Chem.*, **142**, 249 (1942); N. F. Burk, *J. Biol. Chem.* **98**, 333 (1932).

(7) Y. Tonomura, *Kagaku (Science)*, **19**, 565 (1949).

The author wishes to express his hearty thanks to Prof. Bun-ichi Tamamushi, Prof. San-ichiro Mizushima and Dr. Takehiko Shimanouchi for their very kind advice, discussions and encouragement. This work was aided by a grant from the Ministry of Education.

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The Structure of Surface-Denatured Protein. II. Relation between the Surface Diffusion Constant and the Shape of the Protein Molecule (Theoretical)

By Kazutomo IMAHORI

(Received July 2, 1951)

In the previous paper of this series[1], the molecular weight of the surface-denatured serum albumin has been obtained: 70,000 from the $FA-F$ curve. The surface area of the serum albumin is also obtained: 9820 Å.^{2} from the compressibility measurement. Now it is desired to clarify the shape of the surface-denatured protein.

The shape of the native protein molecule has already been investigated by the measurement of the viscosity of the solution, determination of its diffusion constant, measurement of the sedimentation equilibrium under the ultracentrifugal force, etc.

The shape of the surface-denatured protein is likely to be determined by the analogous experiment. Of those methods the measurement of the viscosity and the diffusion constant are applicable for the surface film.

In our laboratory the latter method has been carried on. In this paper the relation between the surface diffusion constant and the shape of the molecule will be derived theoretically.

Relation between the Diffusion Constant and the Friction Ratio

It is perhaps quite natural to postulate that the surface-denatured protein molecule has the form of an elliptic disk, which floats upon the water surface. Suppose at first the disk is circular.

If the relative speed of the disk and water be taken as U, then the frictional force K can be given as

$$K=6\pi\eta p'U \tag{1}$$

where, η is the viscosity coefficient of water. According to Lamb[2], p' is expressed as $8r/9\pi$, r being the radius of the disk. The driving force acting on the protein molecules which are contained in 1 cm.^{2} of area can be expressed as $-\mathrm{d}F/\mathrm{d}x$, where F is the surface pressure of protein film. One square centimeter of area containe $(\rho/M)\times N$ protein molecules, in which ρ is the mass of protein contained in 1 cm.^{2} of area, M is the molecular weight of protein, and N is the Avogadro's number. Thus, the driving force acting on one protein molecule can be shown as $-(M/\rho N)(\mathrm{d}F/\mathrm{d}x)$. This force must be equal to K in magnitude and must have the opposite sign to that of K. When surface film is gaseous we can put

$$F=\frac{R}{M}\rho T \tag{1'}$$

so that $\dfrac{\mathrm{d}F}{\mathrm{d}x}=\dfrac{R\cdot T}{M}\cdot\dfrac{\mathrm{d}\rho}{\mathrm{d}x}$ must hold, and finally we get

$$6\pi\eta p'U=\frac{RT}{\rho N}\frac{\mathrm{d}\rho}{\mathrm{d}x} \tag{2}$$

or

$$U=\frac{R\cdot T}{6\pi\eta\rho Np'}\frac{\mathrm{d}\rho}{\mathrm{d}x} \tag{2'}$$

The mass of protein which diffuses in one second across a line of 1 cm. long will be expressed as $U\rho$. On the other hand from Eq.

(1) K. Imahori, This Bulletin, **25**, 7 (1952).

(2) H. Lamb, "Hydrodynamics," 6th edition, Cambridge, 1932, p. 605.

(2) this can be expressed as follows:

$$U\rho = -\frac{RT}{6\pi\eta N p'}\frac{d\rho}{dx} \tag{4}$$

As mentioned above, $p' = 8r/9\pi$, and the Eq. (4) may be expressed:

$$U\rho = -\frac{3RT}{16\eta N r}\frac{d\rho}{dx} \tag{4'}$$

The diffusion constant D_0 can be defined as

$$U\rho = -D_0\frac{d\rho}{dx} \tag{5}$$

so that from Eqs. (4') and (5) the following relation will be obtained:

$$D_0 = \frac{3RT}{16\eta}\frac{1}{N\cdot r} \tag{6}$$

The suffix 0 means that the molecule is circular.

As $16\eta r/3$ means the frictional force acting on the circular disk when it is floating at a unit speed, we can put

$$\frac{16\eta r}{3} = f_0$$

Then (6) reduces to:

$$D_0 = \frac{R\cdot T}{N}\cdot\frac{1}{f_0} \tag{7}$$

When the disk is not circular, but elliptic, the frictional force may be expressed as f, and just like for the circular disk, the next relation can be obtained for the elliptic disk:

$$D = \frac{R\cdot T}{N}\frac{1}{f} \tag{8}$$

From (7) and (8) is obtained:

$$\frac{D}{D_0} = \frac{f_0}{f} \tag{9}$$

Now, the molecular weight M of a circular disk-shaped protein molecule can be expressed by r (the radius of disk), d (its thickness), V (the reciprocal of the density) and N (Avogadro's number), as follows

$$M = \pi r^2 d\frac{N}{V} \tag{10}$$

Combining with (6) and (9), (10) can be expressed as

$$M = \frac{\pi d N}{V}\cdot\left(\frac{3}{16\eta}\right)^2\cdot\left(\frac{RT}{ND_0}\right)^2 = \frac{9\pi d R^2T^2}{256\,\eta^2 N D^2 V}\left(\frac{f_0}{f}\right)^2 \tag{11}$$

This shows the relation between the diffusion constant D and the friction ratio f/f_0. In the equation, M can be determined by the method described in the previous paper[1]. The thickness d can be determined by the optical method which will be reported in the later paper[3]. And thus f/f_0 can be determined from equation (11), if the value of D is known. The method for determination of the value of D will be reported in part 3 of this series.

Relation between the Friction Ratio f/f_0, and the Ratio of Two Axes of Ellipse c/a

The friction ratio can be determined from the above equation (11). But in order to determine the actual shape of the ellipse, the relation between f/f_0 and c/a must be derived. In this section, this relation has been derived as follows.

The frictional force acting on the disk which floats on the water surface is supposed to be just a half of the force acting on the disk which moves through the water in its edgeways direction. And when the elliptic disk flows on the water surface, the frictional force acting upon the disk is expressed by

$$K = 6\pi\eta RU$$

where U is the relative velocity of the disk against water, η is the viscosity of water and R is the function of the magnitudes of two axes of the ellipse.

According to Lamb[2], for the movement in its a-axis direction, R can be expressed as R_a and is given by the relation:

$$R_a = \frac{4}{3}\frac{ac}{\chi_0 + \alpha_0 a^2} \tag{13}$$

and

$$\chi_0 = ac\int_0^\infty \frac{d\lambda}{\sqrt{(a^2+\lambda)(c^2+\lambda)\lambda}} \tag{14}$$

$$\alpha_0 = ac\int_0^\infty \frac{d\lambda}{\sqrt{(a^2+\lambda)^3(c^2+\lambda)\lambda}} \tag{15}$$

The former integration can be carried out by setting $\lambda = t^2$

(3) K. Imahori and Y. Yonevama, unpublished.

$$\int_0^\infty \frac{d\lambda}{\sqrt{(a^2+\lambda)(c^2+\lambda)\lambda}} = \int_0^\infty \frac{1}{t\sqrt{(a^2+t^2)(c^2+t^2)}} \cdot \frac{d\lambda}{dt} \cdot dt = \int_0^\infty \frac{2dt}{\sqrt{(a^2+t^2)(c^2+t^2)}} = \frac{2}{c} K(e) \tag{16}$$

$K(e)$ means the complete elliptic integral of the first order and e is the eccentricity of the ellipse as given by the following relation:

$$e^2 = \frac{c^2-a^2}{c^2}$$

The integration of α_0 can be carried out as follows. As in the case of X_0, $\lambda=t^2$ is put in (14)

$$\alpha_0 = \int_0^\infty \frac{d\lambda}{\sqrt{(a^2+\lambda)^3(c^2+\lambda)\lambda}} = \int_0^\infty \frac{2dt}{\sqrt{(a^2+t^2)^3(c^2+t^2)}} = \frac{2}{c^2-a^2}\left[\int_0^\infty \sqrt{\frac{(c^2+t^2)}{(a^2+t^2)}}\, dt - \int_0^\infty \frac{dt}{\sqrt{(a^2+t^2)(c^2+t^2)}}\right]$$

The second term in the bracket is just equal to the integration of Eq. (15) and can be given as the complete elliptic integration of the first kind. The former term is the one of the modification of the complete elliptic integral of the second kind. And thus α_0 can be expressed as follows:

$$\alpha_0 = \frac{2}{c^2-a^2}\left[\frac{c}{a^2} \cdot E(e) - \frac{1}{c} \cdot K(e)\right] = \frac{2}{a^2c(c^2-a^2)}[c^2E(e)-a^2K(e)] \tag{17}$$

Putting (16) and (17) into (13), the following will be obtained

$$R_a = \frac{4}{3}\,\frac{1}{\frac{2}{c} \cdot K(e) + \frac{2}{c(c^2-a^2)}[c^2E(e)-a^2K(e)]} = \frac{2}{3}\,\frac{c}{K(e)+\frac{1}{c^2-a^2}[c^2E(e)-a^2K(e)]} \tag{18}$$

And the frictional force acting on the disk can be given from equation (12) as:

$$K_a = \frac{4\pi\eta Uc}{K(e)=\frac{1}{c^2-a^2}[c^2E(e)-a^2K(e)]} \tag{19}$$

In the similar way for the movement in b-axis direction the frictional force K_b can be given as:

$$K_b = \frac{4\pi\eta Uc}{K(e)+\frac{1}{e^2}[K(e)-E(e)]} \tag{20}$$

The molecule on the surface moves quite at random, so frictional force must be averaged statistically for all directions. This can be done approximately by the following relation:

$$\frac{1}{K} = \frac{1}{2K_a} + \frac{1}{2K_b} \tag{21}$$

By putting (19) and (20) into (21), K can be expressed as

$$K = \frac{8\pi\eta Uc}{3K(e)} \tag{22}$$

while, as mentioned above, f_0 can be defined by

$$f_0 = \frac{16\eta r}{3} \tag{23}$$

Now, similarly f can be defined by

$$f = \frac{8\pi\eta c}{3K(e)} \tag{24}$$

This defininition is quite logical, because if the relation $c=a=r$ holds, f reduces to f_0, or when the disk is circular f becomes just equal to f_0.

From (23) and (24) and also by using the relation of

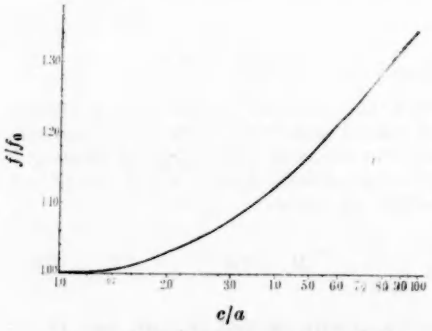

Fig. 1.—Relation between f/f_0 and c/a.

$$ac=r^2$$

the friction ratio is expressed as follows:

$$\frac{f}{f_0}=\frac{\pi}{2K(e)}\cdot\frac{c}{r}=\frac{\pi}{2K(e)}\sqrt{\frac{c}{a}}$$

$$=\frac{\pi}{2K\left(\sqrt{\frac{c^2-a^2}{c^2}}\right)}\cdot\sqrt{\frac{c}{a}} \qquad (25)$$

This shows the relation between f/f_0 and c/a. This relation can be expressed as Fig. 1, and the value of c/a can be derived when the value of f/f_0 is found by the method mentioned above.

Summary

1. The relation between the friction ratio and the surface-diffusion constant has been derived.

2. The relation between the friction ratio and the ratio of two axes of an ellipse has been derived.

3. The relation between f/f_0 and c/a has been shown in a curve which makes the estimation of c/a very simple.

The author wishes to express his best thanks to Prof. Bun-ichi Tamamushi, Prof. San-ichiro Mizushima and Dr. Takehiko Shimanouchi for their kind advice and encouragement. The author also owes much to Professor Isao Imai for helping the hydrodynamical calculation.

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The Structure of Surface-Denatured Protein. III. Determination of the Shape of Surface-Denatured Horse Serum Albumin

By Kazutomo IMAHORI

(Received July 24, 1951)

In the first paper[1] of this series, the molecular weight and the surface area covered by one protein molecule were reported. In the second paper,[2] theoretical treatment on the relation between the shape of the protein molecule and the surface diffusion constant has been given.

In the present paper, the method of calculating the surface-diffusion constant from the measurement of surface potential as well as its result will be reported.

Apparatus of Surface Potential Measurement

There are two methods of measurement of surface potential. One is the so-called vibrating plate method,[3] by Zisman, and the other is the ionization method adopted by Rideal and others.[4] Recently, several chemists in this country have constructed the apparatus which in all cases is of the former type. The present author has constructed one of the latter type, principally because the latter type is superior to the former in the experiment of surface diffusion, as the electrode of the latter type is so thin that the surface potential can be measured from point precisely. The apparatus is shown in Fig. 1.

In the figure, T is the trough, filled with water, upon which the protein film under investigation is spread. The trough is held on a bakelite stand, which is insulated from the earth electrically. The potential of T can be varied by potentiometer P, which is connected with T through caromel cell C and salt junction J. A is an air electrode —made of platinum wire of about 1 mm. diameter and coated with polonium. The polonium ionizes the air between A and T and conncets A electrically with the top of the surface film. A is supported by a pyrex glass bar which is fixed on a steel stand and can be freely moved vertically and horizontally. U is a vaccum tube, UX 54 made by the Tokyo Shibaura Electric Co. Ltd., which has a very large grid resistance ($10^{10}\Omega$). The filament current is 200 mA. R_1, R_2 and R_3 are all variable resistances of 25 Ω. R_6 and R_6' are also variable resistances of 500 kΩ and 100 Ω respectively. R_g is also a variable resistance, the value of which is just equal to the critical damping

(1) K. Imahori, This Bulletin, **25**, 7 (1952).
(2) K. Imahori, This Bulletin, **25**, 10 (1952).
(3) W. A. Zisman, *Rev. Sci. Instr.*, **3**, 367 (1932).
(4) J. R. Schulman and E. K. Rideal, *Proc. Roy. Soc.*, **130, A**, 259 (1931).

reistance of the galvanometer used, and equal to 1 kΩ in the present case.

R_a anc R_b are fixed resistances of 20 kΩ and 1 kΩ respectively. I_f is a milliammeter having a scale up to 500 mA. and G a galvanometer, sensitivity of which is about 2×10^{-8} A. The apparatus must be completely shielded electrically. The operation of this apparatus is as follows.

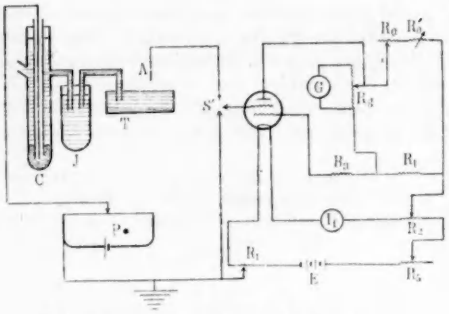


Fig. 1.—Apparatus for measuring the surface potential.

First the switch S in Fig. 1 is put downward and the grid is earthed. By adjusting R_0 and R_0', the needle of G is set to its zero point. Then A is brought about 1~2 mm. above the cleaned surface of the water contained in the trough T. S is switched upward and A is connected to the grid. The needle of the G moves then far from zero point and it is brought back again to near zero point by adjusting P. Now, S is put downward, then upward again and P is adjusted and this operation is repeated several times until the needle of G does not move by this operation. The value, which P indicates, is just equal to the contact potential between A and the water surface and can be expressed by V_1.

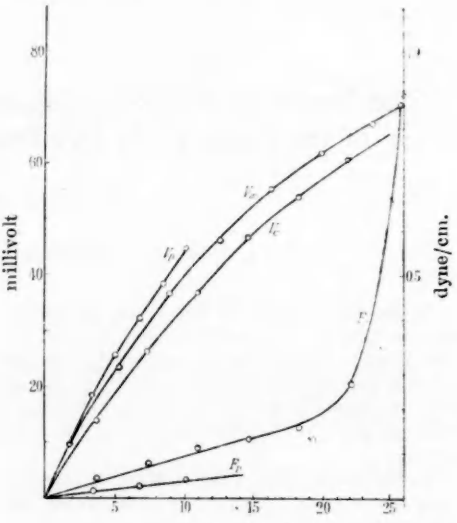
Then monomolecular film of protein is put on the water surface. The contact potential between A and water carrying the protein film can be measured by the similar way and is expressed as V_2. The surface potential ΔV of the protein film is equal to the difference of V_2 and V_1, or

$$\Delta V = V_2 - V_1.$$

The Relation between Concentration and Surface Potential

In the experiment of the surface diffusion constant, it is needful to measure the concentration of protein on the surface. There are probably only a few ways to measure this concentration. The present author has done this from the measurement of surface potential, because, in the region of low concentration, surface potential is thought to be proportional to surface concentration. This can be ascertained from the following experiments. The curve V_P in Fig, 2 shows the relation between the surface potential and the surface cencentration of serum albumin, while the curve F_P shows the relation between the surface pressure and the surface concentration. From these two curves it becomes evident that the surface potential is proportional to the surface concentration in the region of concentration below 0.1 mg./m.^{2} or in the region of surface pressure below 0.05 dyne/cm. Similarly, curves V_c and F_c show that the surface potential of castor oil monolayer is proportional to the surface concentration in the region of surface pressure below 0.5 dyne/cm.

When the mixed film of castor oil and protein monolayer is expanded on the surface, the surface potential varies according to the curve V_m in Fig. 2, where the amount of protein used is constant——2.085×10^{-7}g., while the amount of castor oil is varied.



$\times10^{-2}$ mg./m.^{2}

Fig. 2.

From this curve it is also evident that the surface potential of the mixed film becomes just the sum of two partial surface potentials, namely, the surface potentials of two components expanded on the same surface area, each alone respectively. This shows that there is very little interaction between molecules of protein and castor oil on the surface, under very low surface pressure.

Mesurement of the Surface Diffusion Constant

As is shown in Fig. 1, a tray of 60 cm. long,

20cm. broad, and 4cm. deep, is filled with distilled water or other aqueous substrates. The surface of the water is cleaned with a moving barrier (M and M' in Fig. 3) and then is divided into two

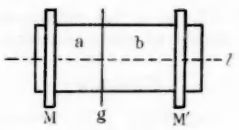


Fig. 3.

parts by a very thin glass rod (g), which is so thin and so light that it can be pushed away by even the slightest surface pressure. The contact potential V_1 of the water surface and the electrode is measured by the apparatus mentioned above. Serum albumin solution in water of 0.0209% concentration is put on one side (side a), of the water surfece, from a micrometer syringe, which makes the gaseous film of serum albumin, and the rod g is pushed to the other side (side b). The sample of serum albumin is just the same as that used in the first paper of this work.

Then a few drops of very dilute benzene (or ethylether) solution of castor oil are put on both sides of the rod until the surface pressures of the both sides balance with each other and the rod g comes to an equilibrium position. The balanced surface pressure must be less than 0.5 dyne/cm.

The air electrode A is carried along the line l of Fig. 3. The surface potential along l can be

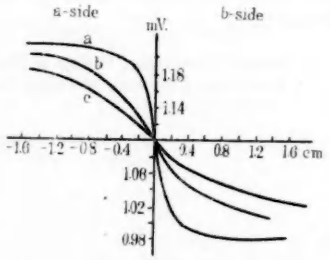


Fig. 4.—Surface potential curve.

shown schematically as the curve a in Fig. 4, in which the abscissa shows the the distance along l. The zero point corresponds with the point where the glass rod crosses the line l. The glass rod must be kept perpendicular to l.

Now, the glass rod is taken away from the water surface and the protein begins to diffuse into the b-side. If, however, castor oil had not been put on the b-side, the protein molecule would flow on the water surface just as a gas flows from a vessel of higher pressure into one of lower pressure, and soon all the surface will be covered by a homogeneous protein film. The velocity of such flow would not mean the true surface diffusion velocity. To avoid the error from this flow movement, some substance (such as castor oil) is put on the b-side so as to balance the surface pressures of both sides. And thus, the net diffusion constant on the surface can be determined.

As time elapses, the surface potential gradient at zero point becomes gradually less and less just as is shown schematically by the curves b and c in Fig. 4.

As is cited above, the experiment is carried out at low surface pressure, and under this condition the potential gradients at various points are proportional to the gradients of the concentration of protein molecules, just as is shown in the above section. The surface potential along l after 24 hours of diffusion is shown in Fig. 5 and the gradients at various points of this curve are shown in Fig. 6. The measurement can be carried out within the error of 0.5 mV.

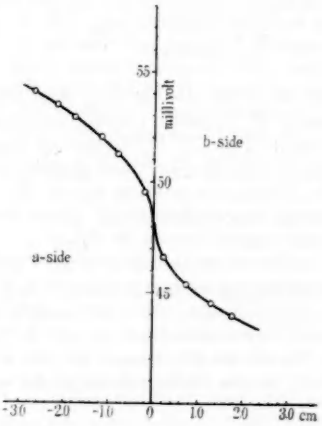


Fig. 5.

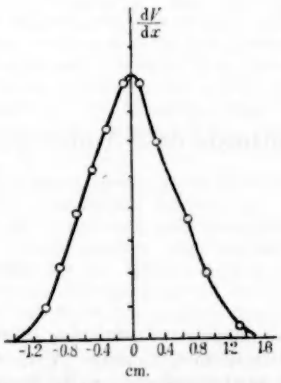


Fig. 6.—Surface potential gradient.

The curve in Fig. 6 also represents the gradients of the concentration of protein on

the surface at various points, so that, just as in the case of three-dimensional diffusion, the diffusion constant can be calculated by the inflection point method. If the distance from the zero point to the inflection point is expressed as μ and the time elapsed from the initiation of the diffusion to the measurement is expressed by t, the diffusion constant can be given as

$$D = \frac{\mu^2}{2t} \qquad (1)$$

From Fig. 6 and Eq. (1), and also by using the data of $\mu = 0.14$ cm., D is calculated to be 1.1×10^{-6} cm.^{2}/sec. By putting this value into Eq. (11) given in the second report of this investigation,

$$M = \frac{9dR^2T^2}{256\eta^2ND^2}(f_0/f)^2$$

and also by using the data, V (reciprocal of the density of protein) $= 0.748$,[5] d (thickness of the film) $= 10$ Å.,[6] M (molecular weight of protein) $= 70{,}400$,[1] f/f_0 (friction ratio) is given as 1.28. Using the relation shown in Fig. 1 of the same report mentioned above, the ratio of two axes turns out to be 8.0.

As was shown in the first report, the surface area covered by one serum albumin molecule is 9800 Å.^{2} and thus the length of the two axes are calculated as: $a = 20$ Å. and $c = 160$ Å. Therefore, the shape of the surface-denatured serum albumin molecule will be schematically expressed as in Fig. 7.

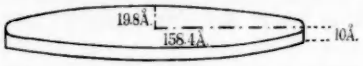


Fig. 7.—Shape of the surface-denatured serum albumin molecule.

(5) T. Svedberg, "Ultracentrifuge," 1940.
(6) K. Imahori and Y. Yoneyama, unpublished.

Summary

1. The apparatus for measuring surface potential by the so-called ionization method has been described.

2. The relation between the surface potential and concentration of serum albumin and castor oil monolayers have been obtained.

3. The method for determining the shape of surface-denatured protein molecules has been described. This can be carried out on the grounds of the determination of surface-diffusion constant by the surface potential measurement.

4. The shape of horse serum albumin has been decided to be an elliptic disk having the value of $a = 20$ Å., $c = 160$ Å. and thickness of 10 Å.

The author wishes to express his hearty thanks to Prof. Henry B. Bull, Prof. Bun-ichi Tamamushi, Prof. San-ichiro Mizushima and Dr. Takehiko Shimanouchi for their kind encouragement and advice. Thir experiment is aided by a Grant in sid for Fundamental Scientific Research defrayed from the Ministry of Education.

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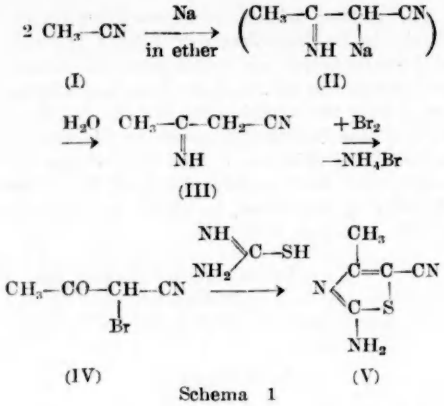
Synthesis of 2-Amino-4-methyl-5-cyanothiazole from Acetonitrile

By Kiku MURATA

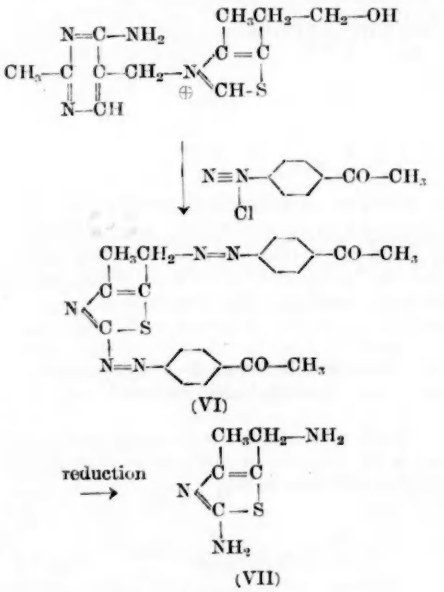
(Received August 14, 1951)

For the purpose of obtaining 2-amino-4-methyl-5-aminomethylthiazole (VII) which is presumed as the substance to be produced by reduction of 2-(*p*-acetylphenylazo)-4-methyl-5-(*p*-acetylphenylazomethyl)-thiazole (VI) believed[1],[2],[3] to be an azo-color from thiamine as shown in Schema 2, a new thiazole, 2-amino-4-methyl-5-cyanothiazole (V) was synthesized from acetonitrile (I) according to the processes shown in Schema 1,

(1) K. Murata, *J. Chem. Soc. Japan*, **64**, 1379 (1943).
(2) K. Murata, *J. Chem. Soc. Japan*, **66**, 57 (1945).
(3) K. Murata, *J. Chem. Soc. Japan*, **66**, 59 (1945).



Schema 1



Schema 2

Catalytic reduction of 2-amino-4-methyl-5-cyanothiazole (V) is being carried on now under several conditions, but it is not yet completed, so the synthesis of this thiazole (V) is reported here.

Experimental

(1) β-Iminobutyronitrile (III).—The preparation of this substance was carried out according to Hortzwart[4]. A three-necked flask of 500 cc. content, equipped with a sealed stirrer, dropping funnel and reflux condenser under the protection of the moisture was used. Metallic sodium (8 g.) in small pieces were put in 300 cc. of absolute ether in the flask, and 25 g. of pure acetonitrile were gradually added with thorough stirring. After all of the metal had dissolved, the flask was warmed for 1 hour at 40° on a water-bath. The precipitates were separated from ether. The crude precipitates (30 g.) presumed as sodio-β-iminobutyronitrile (II) were dissolved in a little water (*ca.* 10 cc.), to which 50 cc. of ether were added and boiled on the water-bath or shaken with ether and water. Then the ether solution was evaporated to one third of its original volume. β-Iminobutyronitrile crystallized from ether after standing for 2 or 3 days in the ice chest. The crude crystals (m. p. 47—65°) (1 g.) were receystallized from benzene at room temperature. The stable form[4],[5]. (m. p. 47—48°) was obtained as crystals soluble in benzene and the labile form[6] (m. p. 74—78°) was obtained from the insoluble part, in yields of 0.3 g. and 0.1 g. respectively. When 4 g. of crystals, m. p. 47—80°, were boiled with benzene for 30 min. on the water-bath, the crystals, m. p. 46—60°, was obtained. When this crystal was boiled again in benzene, the crystals, m. p. 47—48°, separated out in a yield of 0.3 g.

Both specimens, m. p. 47—48° and 46—60°, gave the oxime, m. p. 98—100° (Found: C, 49.26; H, 6.45%) and the semicarbazone, m. p. 159—160° (Found: C, 42.97; H, 6.05%) of β-iminobutyronitrile as described in the literature[7].

(2) α-Bromo-β-ketobutyronitrile (IV).—Nineteen g. of bromine, *i. e.* theoretical amount, were dissolved in 200 cc. of ether and the solution was dropped into 400 cc. of ether solution containing 10 g. of β-iminobutyronitrile (m. p. 47—65° or 50—70°) cooled in an ice bath. The excess of bromine in the ether solution was removed by the treatment with aqueous solution of sodium bisulfite, and the solution was dried with sodium sulfate and evaporated. The resulting oil was separated from ammonium bromide and destilled in vacuum. α-Bromo-β-ketobutyronitrile, b. p. 70—73°/4 mm., was obtained in a yield of about 60% (Found; C, 29.94; H, 2.93%. Calcd. for C_4H_4ON Br: C, 29.64; H 2.47%.) The ammonium bromide recovered amounted to 70% of the theory.

(3) 2-Amino-4-methyl-5-cyanothiazole (V).—When the theoretical amount of powdered thiourea was mixed with α-bromo-β-ketobutyronitrile in water solution and warmed on the water-bath at 80°, the reaction went on gradually. The whole was allowed to stand overnight, and then dissolved in 10 cc. of water. After removing the unreacted substances by ether, the aqueous solution was fitlered and 2 *N* alkali solution (about 15 cc.) was added to the filtrate until *p*H 6.8. The precipitated substance was collected. The

(4) A. Holtzwart, *J. prakt. Chem.*, (2) **39**, 230 (1889).

(5) E. Meyer, *J. prakt. Chem.*, (2) **52**, 84 (1895).

(6) E. Meyer, *J. prakt. Chem.*, (2) **52**, 83 (1895).

(7) E. Meyer, *J. prakt. Chem.*, (2) **78**, 504 (1908).

crystallization from water gave 2-amino-4-methyl-5-cyanothiazole, colorless needles, m. p. 164—166°, easily soluble in benzene, alcohol and hot water, 50% yield. (Found: C, 43.40; H 3.95%. Calcd. for $C_5H_5N_3S$: C, 43.17; H 3.53%.)

Summary

A new thiazole, 2-amino-4-methyl-5-cyanothiazole (V) m. p. 164—166° was synthesized from thiourea and α-bromo-β-ketobutyronitrile (IV), b. p. 70—73°/4 mm., prepared by the action of bromine on β-iminobutyronitrile obtained from acetonitrile.

The author wishes to express her sincere thanks to Dr. Matsukawa of Takeda Institute who kindly helped her in this research. Thanks are also due to Mr. Ikehata who has assisted her during the experiments, and to Dr. Chachin of this Research Institute for his support in executing this work. The cost of this research has been partly defrayed from the Ministry of Education, to which the author's thanks are due.

Osaka Municipal Institute for Hygiene, Osaka

On the Properties of 1-Thiol-benzthiazole Derivatives as Photographic Addition Agents

By Yasushi OH-YAMA

(Received August 16, 1951)

Introduction

Previously the author reported several times about the derivatives of 2-mercapto-benzimidazole as addition agents for photographic emulsions and developers.[1],[2],[3],[4]

In this connection he has been examining from that time the probabilities or the properties as additions of 1-thiol-benzthiazole, usually called 2-mercapto-benzthiazole, intimately related compound of the above mentioned, and soon the practical importance of this compound in photography has been recognized.

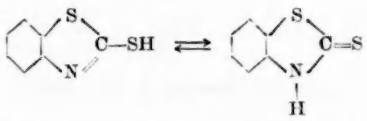
It is interesting to know that the usefulness of this compound has been recently disclosed in other countries.[5],[6]

The author's intention, being interrupted with the short supply of materials during the war, to prepare and examine those derivatives of it, as were derived of 2-mercapto-benzimidazole, is now attained.

In this report, properties of two kinds of alkyl (and aralkyl) derivatives of this compound are described as additions in developer for pure silver chloride emulsion and as additions in emulsion for chloride and chloro-bromide emulsions and compared with similar derivatives of mercapto-benzimidazole already reported.

Relation between S-Alkyl- and N-Alkyl-derivatives

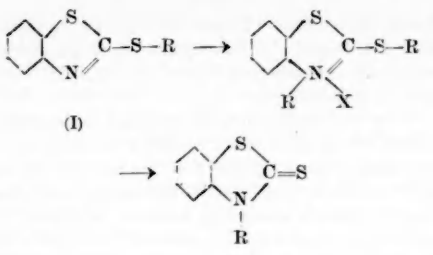
1-Thiol-benzthiazole is a single substance having two tautomeric configurations by proton transfer as shown below,



but those compounds, in which this replaceable hydrogen atom is substituted by alkyl (or aralkyl) radical, form two distinct compounds, S-alkyl-derivatives (I) and N-alkyl-derivatives (II), each must be called 1-alkylthio-benzthiazole (I) and 1-thio-2-alkyl-1:2-dihydro-benzthiazole (II), and it is not easy to convert one to the other.

Generally (II) is more stable than its corresponding compound of (I), and (I) can be converted to (II) through its quaternary ammonium salt

(1) Yasushi Oh-yama, *Bull. Inst. Phys. Chem. Research (in Japanese)*, **22**, 483 (1943).
(2) Yasushi Oh-yama, *ibid.*, **23**, Chem. 7 (1944).
(3) Yasushi Oh-yama, *J. Soc. Scientific Photography Japan (in Japanese)*, **9**, 1 (1943).
(4) Yasushi Oh-yama, *ibid.*, **9**, 57 (1943).
(5) A. Bycichin and L. Vlach, *Chem. Listy*, **41**, 136; *Sci. Ind. Phot.*, **18**, 33; *C. A.* **42**, 1518 (1948).
(6) W. H. Dimsdale and R. R. Robinson (Ilford Ltd.), U. S. P. 2, 432, 864; U. S. P. 2, 432, 865.



(I)

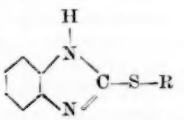
(II)

or by other means. And it is also possible to change its alkyl radical on this occasion.[7]~[10]

Experimental

In homologues of S-alkylthio-benzthiazole (I), methyl, ethyl, allyl, benzyl and carboxymethyl-derivatives, and in those of N-alkyl-compounds (II), methyl, ethyl, allyl[11] and benzyl derivatives were prepared and tested.

The similar S-alkyl-derivatives (III) of mercapto-benzimidazole



(III)

(*i. e.*, 2-thiol-benzimidazole), which were previously reported[1],[3], were tested in comparison with these compounds.

Strips of specially made gaslight paper which was coated with pure silver chloride-gelatin emulsion, were exposed at a fixed condition under optical wedge, developed 1 min. at 20°C., in an ordinary developer containing metol, hydroquinone, sodium sulphite, sodium carbonate but not potassium bromide and finally added varied amounts of above mentioned compounds dissolved in methanol (0.1~1%).

The relation between the time of appearance of images, the fog, the tone of images and the amount of substances added were examined.

The emulsion tests of these compounds were also carried out in some case of gaslight and chloro-bromide emulsions.

Results

Results of above experiments were illustrated in Fig. 1, Fig. 2 and Table 1:

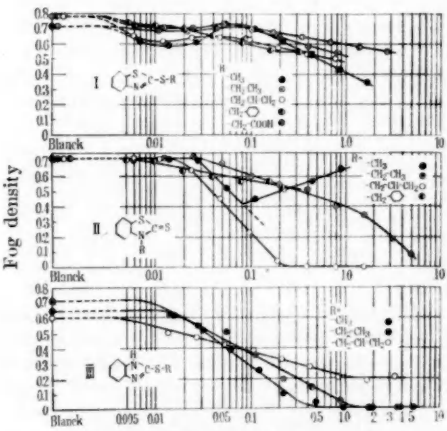


Fig. 1.—Relation between fog-density and concentration of addition in developer.

Table 1

Blue-black Developing Property of Derivatives of 1-Thiol-benzthiazole and 2-Thiol-benzimidazole

Substance added in developer	Minimum conc. to produce appreciable effect	Concentration to produce maximum effect	Bluishness of tone
1-Methylthio-denzthiazole	$0.027 < C < 0.055$	0.44 (approx.)	+
1-Ethylthio- 〃	$0.025 < C < 0.051$	0.20~0.40	+
1-Allylthio- 〃	0.024 (approx.)	0.39 (approx.)	++
1-Benzylthio- 〃	0.32 (approx.)	?	very weak
1-Thio-2-methyl-1:2-dehydro-benzthiazole	$0.027 < C < 0.055$	0.44 (approx.)	++++
1-Thio-2-ethyl-1:2- 〃	$1.60 < C < 3.20$	?	++
1-Thio-2-allyl-1:2- 〃	$0.006 < C < 0.012$	0.77 (approx.)	+++
1-Thio-2-benzyl-1:2- 〃	0.08 (approx.)	0.16 (〃)	very weak
2-Methylthio-benzimidazole	$0.015 < C < 0.030$	0.48 (approx.)	+
2-Ethylthio- 〃	$C < 0.014$	0.45 (〃)	+
2-Allylthio- 〃	$C < 0.013$	0.42 (〃)	+++

(7) C. Hasan and R. F. Hunter, *J. Chem. Soc.*, **1936**, 1672.
(8) J. D. Kendall (Ilford Ltd.), F. P. 821, 980; B. P. 475, 647.
(9) W. A. Sexton, *J. Chem. Soc.*, **1939**, 470.
(10) F. P. Reed, A. Robertson and W. A. Sexton, *J. Chem. Soc.*; **1939**, 473.
(11) Melting point of this compound (1-thio-2-allyl-1:2-dihydro-benzthiazole) is 66.5~67.0°C.

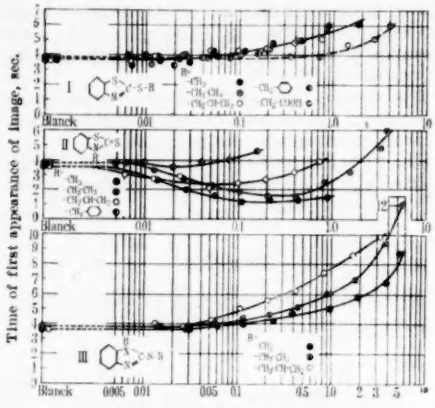


Fig. 2.—Relation between time of first appearance of image and concentration of addition.

Behaviors of these additions when added in emulsion, though these tests were not carried out systematically, keep parallel with those tendency they presented when added in developer. And it would be able to conclude as follows.

(A) Anti-fogging or Fogging Properties.—All of these compounds have fog-preventing or fog reducing property. Generally most of N-alkyl compounds (II) of 1-thiol-benzthiazole surpass even the marked fog-preventing property of any of the S-alkyl derivatives of 2-thiol-benzimidazole (III), on the contrary the S-alkylthio-benzthiazole (I) are inferior compared with the above mentioned and almost equal with its parent substance (1-thiol-benzthiazole).

The only exception is the methyl derivative of (II), which exhibits fog-preventing property at low concentration in developer and causes a considerable degree of fog at high concentration, and this tendency can also be seen when added in emulsion. The emulsion which contains such an amount of it that restrains fog when prepared, sometimes becomes heavily fogged during the storage or incubation test.

The ethyl or allyl derivative of (II) is not only good as a fog-reducer in developer, but can give the emulsions of very good durability. Similarly the benzyl derivative which cannot exhibit a noticeable effect in developer owing to its low solubility, can restrain fog and increase durability when added in emulsion.

(B) Blue-black-developing Property.—The tone or color of the developed silver image of chloride or chloro-bromide emulsion turns from neutral black or warm-black to blue-black, when most of these derivatives (I, II and III) are added in developer or in emulsion, and this property cannot be seen in their parent substances.

This tendency differs according to the substituents, in (I) and (III) it grows stronger when the alkyl radical is replaced by methyl, ethyl, allyl in turns, but benzyl derivatives of these are very weak probably because of their low solubility, and carboxymethyl ($-CH_2-COOH$) derivatives show no effect.

On the contrary N-methyl, ethyl and allyl derivatives (II) of this dihydro-benzthiazole show equally very strong blue-black developing effect, even the benzyl derivative of it, being very weak in developer, shows fairly good effect when added in emulsion.

(C) Development Accelerating or Retarding Property and Sensitizing or Desensitizing Effect.—Derivatives of (I) and (III) series retard development more or less, and derivatives of (II) series, to the contrary, accelerate it.

Plotting the time of first appearance of image against the concentration in developer of these additions (Fig. 2), it shows characteristic curves for each series (I, II and III) and in series (II) and (III) their effects differ regularly according to the number of carbon atoms in the substituted alkyl radical *e. g.*, methyl, ethyl, allyl, benzyl in turns, while in series (I) they bear no evidence of this difference except the carboxymethyl derivative.

The sensitizing and desensitizing properties of them in emulsions run parallel with their development accelerating and retarding properties, (II) has somewhat sensitizing property for silver chloride emulsion as one of the thions which Kendall[12] already pointed out, and derivatives of series (I) and (III) show rather desensitizing effect but not noticeable in ordinary case.

Discussion

The similarity of actions of (I) and (III) lies in parallel with the resemblance of their constitution, and it is supposed to be attributable to the imino group in the imidazole ring of (III) that it exceeds (I) in fog-preventing property and development retardation.

It is recognized from blue-black developing property of these three series, that the imino radical gives scarcely any effect upon this property and the relation between this property and development retardation is not basic.

The considerable difference between(I)and(II),

(12) J. D. Kendall, IXth. Cong. Phot. 1935, Paris, 235.

which is the influence of the same substituent in different positions of the molecule, is supposed mainly to be attributable to the change of the constitution of the sulfur atom of thio-ether type to more reactive thio-keto type.

It is easy to point out this thio-keto radical as the cause of sensitizing, development-accelerating or fog-preventing property of (II).

The sulfur atom of this radical forms complex with silver or silver salts and the stability, the solubility, the rate of decomposition or the delicate combination of those character of this complex compound will be the cause of complicated, sometimes contradictory behavior of these compounds as additions. The behavior of methyl derivative of the series (II) surely indicate the lack of this stability of the complex compound between the said derivative and silver chloride.

Summary

S-Alkyl-(I) and N-alkyl-derivatives (II) of 1-thiol-benzthiazole are prepared and tested as additions for silver chloride emulsions, and compared with previously reported S-alkyl-derivatives (III) of 2-thiol-benzimidazole. Properties of these substances as additions may differ according to alkyl radicals substituted, positions of substitution and their parent substances.

(1) Most of these compounds (I, II and III) show blue-black developing property which can not be seen in parent substances. This effect is the weakest in (I) and the strongest in (II), and as a whole, being the substituent methyl, ethyl or allyl, its activity rises in turn. Benzyl derivatives of them are almost inactive and carboxymethyl derivatives of (I) and (III) are inactive.

(2) As for the fog-preventing property, (II) and (III) have greater activity and increase activity as the number of carbon atoms in the substituent increases. (I) is not so active as (II) or (III) regardless of its substituents.

(3) It is remrakable that (II) has somewhat sensitizing property added in emulsion, and accelerate the development when both (I) and (III) retard it.

(4) The similarity of actions of (I) and (III) lies in parallel with the resemblance of their chemical constitution, but (I) is inferior to (III) in those properties which are supposed to be due to the imino group in the imidazole ring, as fog-preventing action.

(5) The considerable difference between (I) and (II) which is the influence of the same substituent in different position of the molecule, is supposed to be attributable to the change of the constitution of the sulfur atom of thio-ether type to more reactive thio-keto type. The sensitizing, fog-preventing or development-accelerating action of the latter is supposed to be intimately related to the complex forming character of this thio-keto group with silver or silver salts.

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Kyoto

On the Nature of Built-up Film. I. Adsorption of Ion on Built-up Film of Stearic Acid

By Mitsuo MURAMATSU and Tunetaka SASAKI

(Received September 3, 1951)

Introduction

It has been shown by several investigators that the heavy metallic ions exhibit remarkable effects on the surface properties of monolayer of fatty acid spread on water[1] or multilayer transferred onto a solid surface.[2],[3] That the built-up film of fatty acid on solid shows a high wettability when conditioned with the solution containing polyvalent ion, such as aluminum,[4] thorium,[5] or uranyl ion,[6] and that the protein molecules can be adsorbed on such a conditioned surface also suggest a special action of heavy metallic ion on fatty acid.

(1) I. Langmuir and V. J. Schaefer, *J. Am. Chem. Soc.*, **58**, 284 (1936); **59**, 2400 (1937).
(2) K. B. Blodgett, *J. Phys. Chem.*, **41**, 975 (1937); K. B. Blodgett and I. Langmuir, *Phys. Rev.*, **51**, 964 (1937).
(3) I. Langmuir, "Recent Advances in Surface Chemistry and Chemical Physics", Science Press Printing Co., Lancaster, 1939, p. 1.
(4) I. Langmuir and V. J. Schaefer, *J. Am. Chem. Soc.*, **59**, 1406 (1937).
(5) I. Langmuir and V. J. Schaefer, *J. Am. Chem. Soc.*, **59**, 1762 (1937); **60**, 135 (1938).
(6) G. H. A. Clowes, "Recent Advances in Surface Chemistry and Chemical Physics," Science Press Printing Co., Lancaster, 1939, p. 61.

The experimental data, presented by Clowes[6], which showed that the built-up film adsorbed a maximum amount of protein when it was conditioned with the aqueous uranyl salt solution at *p*H 6.0~6.5, lead us further to the presumption that the metallic ion requires an optimum *p*H range for the adsorption. If we adopt the opinion of Kraus and Nelson, [7] insisting rather a complex nature of uranyl ion in aqueous solution, its adsorption may naturally be of a more complex nature than it appears. The present authors have also intended to study the action of polyvalent ion for the built-up film of stearic acid, concerning such a problem.

Experimental

a. Material.—Stearic acid used was Merck's pure chemical reagent. Barium, aluminum, and mercuric chlorides, copper sulfate, and thorium nitrate were recrystallized repeatedly. Substrate water was made pure enough so as no impurity could be detected from the detail of the pressure~area curve obtained for monolayers of fatty acid spread on it.

b. Method.—Most of the experiments were carried out in the temperature range of 23~28°C., in which, however, the monolayer of stearic acid on mere distilled water tends to expand when the pressure of the piston oil is too low, and collapses when it is high[5]. Since the collapsing effect readily appears (2 min. after the spreading), the echelon consisting of pure stearic acid could hardly be prepared while the monolayer on water is stable. For this reason, the following procedure was adopted. The highly polished stainless steel slide, on which a mixed multilayer of stearic acid and barium stearate was deposited, was immersed in *N*/100 hydrochloric acid in order to convert barium stearate to free fatty acid[9], and this converted layer was covered with a few layers of stearic acid by means of an ordinary technique. Thus, barium stearate could be converted to free acid in the depth of several layers at temperature above 25°C.

To minimize the accidental experimental error, a half part of the slide (called the treated side hereafter) was dipped in a solution containing metallic ion, and the other half (the control side) in a control solution. An apparent increase in thickness due to the adsorption was then determined by means of the optical interference method[10], assuming that the spacing and refractive index of barium stearate are 24.4 Å. per layer and 1.462 respectively[5]. The number of equivalent layers on which the ions were supposed to be compactly adsorbed was determined by the skeletonization with benzene.

The *p*H of the solution was adjusted simply by redistilled hydrochloric acid and aqueous ammonia solution when required.

Experimental Results and Discussion

a. Effect of Acid and Alkali.—When the echelon was treated with various kinds of acid or alkali solutions, an appreciable change in composition generally occurred, frequently the change reaching several layers in the depth. For instance, when the echelon was treated with solution of *p*H<2 for 30 sec., the thickness of echelon decreased, indicating presumably the conversion of barium stearate underneath into free acid. A more remarkable decrease appeared in a strong alkaline region. After treating the control side of the echelon with acid solution of *p*H=2 and the other half with that of varying *p*H, the difference in thickness between both echelons was measured as shown in Table 1. These changes may be ascribed to the dissolution of stearate ion into the bulk of solution.

Table 1

Change in Thickness of Echelon Treated by Acid or Alkali

*p*H	2.0	3.2	3.9	5.0	6.6
Change in thickness, Å.	control	0.0	−0.1	−0.8	−0.3
*p*H	7.3	8.3	8.8	9.0	9.4	9.8
Change in thickness, Å.	+0.4	+0.5	−5.2	−6.8	−34.3	−32.9

b. Adsorption of Various Ions.—The amounts of the adsorption of barium, mercuric, cupric, aluminum, and thorium ions from 10^{-4} mol/l. aqueous solutions of varying *p*H were determined and shown in Fig. 1, where the abscissa denoted *p*H and the ordinate, the amount of adsorption expressed by the difference in thickness (in Å.) between the treated and the control sides of a slide. The figure shows that there exists an optimum *p*H range of adsorption for each metallic ion. The adsorption is usually not remarkable in other region of *p*H, presumably due to the decrease in the concentration either of metallic ion in the solution or of stearate ion on the surface. It can therefore be expected that the optimum range of *p*H for the adsorption depends on the nature of metallic ion concerned.

The apparent increment in the ordinate in Fig. 1 at *p*H exceeding 9 may be accounted

(7) K. A. Kraus and F. Nelson, *J. Am. Chem. Soc.*, **71**, 2510, 2517 (1949).
(8) K. B. Blodgett, *J. Am. Chem. Soc.*, **57**, 1007 (1935).
(9) A. Rothen, *J. Am. Chem. Soc.*, **70**, 2732 (1948).
(10) T. Tachibana, *Seikagaku* (*Biochemistry*, in Japanese), **20**, 8 (1948).

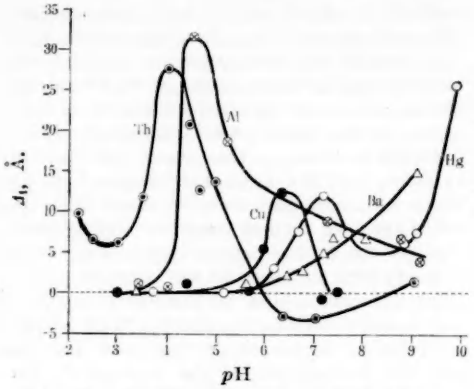

Fig. 1.—Apparent increase (Δ_1) in thickness due to adsorption.

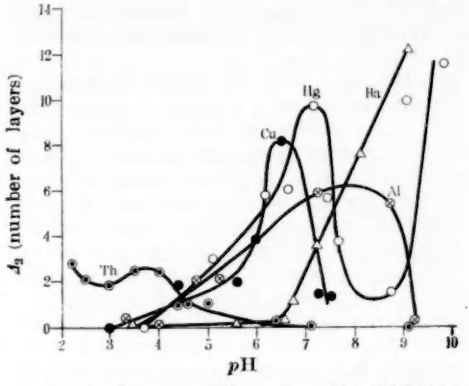

Fig. 2.—Apparent difference (Δ_2) in thickness between treated and control sides of echelon after skeletonization, taking the thickness of barium stearate monolayer as unit.

for by the dissolution of stearate molecules in the multilayers of the control side into the bulk of solution as shown in Table 1, and not by the increase in thickness on the treated side; practically a remarkable decrease was seen on the control side with increasing time of soaking, while only a little increment was observed on the treated side. Barium ion, and perhaps some other ions such as mercuric ion, when they were adsorbed on multilayers, showed a protecting effect against its dissolution into the alkaline medium, just as the effect of these ions on the monolayer spread on water[11].

c. Skeletonization of Adsorbed Layers.—The echelon treated as mentioned above was immersed into benzene for thirty seconds. This procedure brought a very striking difference in thickness between the control and the treated sides. It may be accounted for by the difference in resistance to the dissolution due to the presence of metallic soap on the treated side. Thus the amount of stearic acid which took part in the formation of metallic soap could be estimated by assuming that the metal stearate was practically insoluble in benzene, and that its structure in the multilayer could be preserved after skeletonization. Though it was doubtful whether the experimental conditions were good enough to allow these assumptions, the numbers of stearic acid layers taking part in the adsorption are roughly calculated as shown in Fig. 2. Judging from Figs. 1 and 2, it can be said that the increase in thickness due to the adsorption is rather pronounced in the case of a relatively few position of anchorage for the adsorbing ion. The ratio of the ordinate (Δ_1) of Fig. 1 to that (Δ_2) of Fig. 2 was calculated and illustrated in Fig. 3. This ratio is roughly

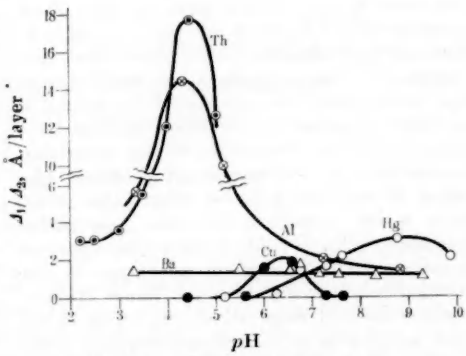

Fig. 3.—Adsorbed amount of ions per layer at various pH.

considered to be the increase in thickness when a stearic acid monolayer is completely changed to metallic soap, and a very specific nature of individual metallic ions can be seen in this figure. Thus, the adsorption of barium ion caused, irrespective of pH, the increment of 1.7 Å. in thickness, which was in a good agreement with that expected from the known spacing of stearic acid and barium stearate, whilst cupric and mercuric ions showed a maximum value of thickness in a specific pH range. It may be added further that one of the above mentioned assumptions could be valid for these three ions, namely, by contacting with vapor of n-octane, the

(11) T. Sasaki and R. Matuura, This Bulletin, **24**, 274 (1951).

skeletonized echelon almost restored the original thickness which it possessed before soaked into benzene.

On the other hand, the values of aluminum and thorium offered a question concerning the limit of the validity of skeletonization. The adsorbed amount per layer shown in Fig. 3 would be too large when compared with the adsorbed amounts of these ions on the multilayers of barium stearate[4),(5)] where it could be considered that the adsorption below a layer did not take place.[12] From the experience of the present authors, it seemed impossible to exceed 3 and 5 Å. in any case for alumiuum and thorium respectively. By contacting with *n*-octane vapor, it could be confirmed that the skeleton collapsed after the skeletonization. Furthermore, it has also been pointed out[2] that the meaning of skeletonization is obscure unless the content of metallic soap in the layer is more than 50 per cents. Therefore, we attempted further to determine the reliable value of the number of stearic acid layers taking part in the formation of metallic soap.

If the adsorption takes place in a few outermost layers of the built-up film, there exists a sandwich layer of pure stearic acid between the adsorbed layer and the base multilayer. As a result, when this echelon was skeletonized, the adsorbed layer would be partially detached from echeion into the solution, due to the dissolution of the underlying sandwich layer of stearic acid. Since this effect is considered to be remarkable when only a few surface layers take part in the adsorption, it is evident that the apparent amount of the adsorption per layer is also exaggerated in such a case. These circumstances are still more likely to occur in the case where a large or complex ion may suffer resistance to the penetration of the surface layers for the deeper adsorption.

These presumption could be confirmed from the following experiment. Instead of the multilayers of stearic acid converted from barium salt, definite multilayers of stearic acid were deposited from water the surface of water onto the barium stearate echelon at 8~12°C., at which the monolayers could be transferred repeatedly without collapse, and the experiments of the adsorption of ion and the successive skeletonization were carried out similarly. The experimental results showed that the amounts of the adsorption were nearly the same as in the former case in all *p*H region, while the detachment of stearate during skeletonization seemed to be fairly remarkable. This may be probable, since the stearic acid in this case can be completely removed by skeletonization, while, in the former case, the stearic acid layer contains some barium stearates which resist the detachment effect mentioned above. We could cite further evidence for this argument. From 2 to 10 layers of stearic acid were transferred onto the multilayers of barium stearate. Using these surfaces the similar experiments were repeated. The apparent amounts of the adsorption per layer as a function of the number of layers of stearic acid initially transferred were shown in Table 2. It was about 2 Å. for barium at *p*H 9.0 independent of the number of the layers. This value may be expected from the spacing of the built-up layers of barium stearate (24.4 Å.) and of stearic acid (23.1 Å.).

Table 2

Apparent Amount of Adsorption as a Function of the Number of Layers of Stearic Acid

No. of layers of stearic acid on echelon	Adsorbed amount per layer in Å.		
	Ba at *p*H 7.4	Ba at *p*H 9.0	Al at *p*H 4.3
2	2.6	1.6	5.0
4	3.2	2.1	3.9
6	27	2.5	20.0
8	35	2.1	52.1
10	50	1.9	68

On the other hand, there was a remarkable increase in thickness of the adsorbed film between 4 and 6 stearic acid layers of aluminum at *p*H 4.3 or for barium at *p*H 7.4. These results seem to show that these ions can penetrate only about 4 layers at the corresponding *p*H. This table does not only suggest the existence of the sandwich effect, but also enables us to calculate the adsorbed amount per layer for aluminum, being about 5 Å., which can be expected from the content (usually 60 ~80%) of barium stearate in the ordinary echelon and increment in thickness (~3 Å.), when it was conditioned with aluminum ion. That the aluminum ion could, even at the most favorable *p*H for the adsorption, penetrate only a few layers, suggests its complex nature as compared with barium ion, which has already been pointed out for instance by McGee[13]. Recently, Gerovich and Kaptsan[14] pointed out the anomalous surface potential of monolayers of stearic acid spread on water

(12) I. Langmuir, *Proc. Roy. Soc.*, **A 170**, 1 (1939).

(13) C. G. McGee, *J. Am. Chem. Soc.*, **71**, 278(1949); *c/c.*

(14) M. Gerovich and O. Kaptsan, *Zhur. Fiz. Khim.*, **23**, 445 (1949); *C. A.* **43**, 6038 (1949).

containing thorium salt at *p*H 2.5. This also seems to have some relation to our experiment. Furthermore, it was reported by Sasaki and Matuura[11] of our laboratory that the monolayers of stearic acid spread on the aqueous solutions containing ions of copper, aluminum, thorium, etc., remarkably expanded at the *p*H ranges corresponding to those of Fig. 3. From the data shown in Figs. 1 to 3, it may also be reasonable to assume more or less large unit of the complicated structure at least in the specific *p*H range for cupric, mercuric, aluminum, and thorium ions in the solution, the degree of complexity being presumably as follows:

thorium ion>aluminum ion>cupric ion.

Summary

The adsorption of barium, mercuric, cupric, aluminum, and thorium ions on the built-up films of stearic acid both transferred from water the surface of water, and converted from barium stearate multilayers, was studied.

The adsorbed amount of barium ion increased with increasing *p*H, but the increase in thickness corresponding to the saturate adsorption for monolayer was constant for all *p*H. On the contrary, other ions studied showed the specific *p*H range for the optimum adsorption. A more or less complicated and large structure for these ions was concluded from the special nature of the adsorption hereby exhibited by these ions. Such a complex nature was most pronounced for aluminum and thorium ions.

The cost of this research has been defrayed from the grant of the Ministry of Education, to which the authors' thanks are due.

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On the Nature of Built-up Film. II. Deposition of Monolayer of Stearic Acid from Surface of Water Containing Copper, Aluminum, and Thorium Ions

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(Received September 3, 1951)

Introduction

The built-up film of stearate can readily be prepared when the substrate water from which it is built up contains barium or calcium ion. If, however, it contains a trace of heavy metallic ion, the nature of this film is profoundly altered.[1] As pointed out by Blodgett,[2] this effect seems to be very remarkable in case of copper ion which hinders the deposition of monolayer on a metallic slide, even if the other conditions are favorable for the successive deposition. She also reported[3] that the monolayer spread on water containing aluminum chloride could not be deposited onto the slide. In the preceding report,[4] the authors were inclined to the opinion that some metallic ions, *e. g.*, cupric, mercuric, aluminum, and thorium ions, somewhat differ in the manner of adsorption from alkali earth ions. They seem to behave rather as a large unit of the complicated structure in a specific *p*H range, at which the adsorption is remarkable. This conclusion has also been supported by the experimental results on monolayer spread on water, carried out by Sasaki and Matuura of our laboratory.[5] In the present report, it was attempted to build up stearic acid monolayers by transferring from the surface of aqueous solutions containing cupric, aluminum, and thorium ions, and the properties of these films were studied.

Experimental

Materials used were purified similarly as describ-

(1) I. Langmuir, "Recent Advances in Surface Chemistry and Chemical Physics," p. 1, Science Press Printing Co., Lancaster (1939); I. Langmuir and V. J. Schaefer, *J. Am. Chem. Soc.*, **58**, 284 (1936); **59**, 2400 (1937).
(2) K. B. Blodgett, *J. Phys. Chem.*, **41**, 975 (1937).
(3) K. B. Blodgett, *J. Am. Chem. Soc.*, **57**, 1007 (1935).
(4) M. Muramatsu and T. Sasaki, This Bulletin, **25**, 21, (1952).
(5) T. Sasaki and R. Matuura, This Bulletin, **24**, 274 (1951).

ed in the preceding report.[4] Stearic acid was spread as benzene solution on the surface of aqueous solutions of copper sulfate (*p*H 6.5), aluminum chloride (*p*H 6.3), and thorium nitrate (*p*H 4.0), *p*H of which was properly adjusted using ammonia and hydrochloric acid. Concentration of salts was 10^{-4} mol/l. The method of deposition will be described in the following section. The spacing of film, transferred onto the echelon of barium stearate, was determined by means of the optical method as in the preceding report.[4] The contact angle was measured by an image method.[6] Thereby we ignored the disturbance due to the evaporation of water, but the results were quite satisfactory for the present purpose.

Results

a. Deposition of Monolayers onto the Slide.—It was confirmed by the preliminary experiments, that the difficulty of transference onto the slide of monolayers of stearic acid spread on aqueous solution containing copper, aluminum, and thorium ions, chiefly comes from their mechanical properties. High rigidity and brittleness spoil the fluidity and flexibility of monolayer which are necessary for building up monolayers. Actually, the piston oil could not exert a uniform pressure on a film. It must be noted further that, in this case, films are so sensitive even for a slight mechanical agitation accompanying dipping and withdrawing of the slide,[1] that the piston oil of high spreading pressure usually employed for this purpose can not be used. Moreover the heavy metal ions in a substrate water seem to influence the nature of an ordinary piston oil, such as castor oil, as it does for stearic acid. Taking into account of these circumstances, the following procedure was adopted to prepare a built-up film. Successive *Y*-multilayers of barium stearate, deposited onto the metal slide, were conditioned with aluminum chloride solution of *p*H 4.3 or with thorium nitrate of *p*H 4.0, and immersed in water containing ion under the investigation. Benzene solution of stearic acid was then applied to that surface of water by a micro-pipette, until only a small drop of benzene remained. The additional drops were put on several positions about 4 cm. apart from the place where the slide was withdrawn. As the slide was withdrawn and monolayer was transferred, drops of benzene spread with the irregular shape, as if they wedge into cracks formed in a rigid surface layer of stearic acid. Here drops behave as a piston oil of the spreading pressure of about 10 dynes per cm. presumably unaffected by a substrate ion. The spreading of drops was the indication of transference of monolayer. It must be noted that, although the first layer of copper, aluminum, and thorium stearates could be built up relatively easily, successive deposition of these films, especially of thorium stearate, occurred in an irregular manner, *i.e.*, we often encountered the folding or the defection of monolayers during its transference from the surface of water.

The first layers of built-up films thus obtained were wettable, to some extent, soon after withdrawal, but became very hydrophobic once they were dried. The contact angle in such a state towards water are shown in Table 1. Here, an *X*-film of barium stearate was built from substrate water of *p*H 9, and the contact angle value was the mean of four to eight experiments.

Table 1

Contact Angles of Built-up Films towards Water

Lower layer	45*Y* Ba-st.		47*Y* Ba-st.		
Upper layer	2*Y* Ba-st.	3*X* Ba-st.	1*B* Cu-st.	1*B* Al-st.	1*B* Th-st.
Contact angle, degree	99±12	102±10	104±11	106±10	110±6

Further the calculation was made for the apparent spacing of monolayers of these built-up films, using the value of 24.4 A. for the spacing of barium stearate monolayer and refractive index of 1.462 which was assumed to be equal to that of base echelon. The result was shown in Table 2. The value of spacing was obtained from the results of five to seven experiments.

Table 2

Apparent Spacing of Copper, Aluminum, and Thorium Stearates

Film	Apparent spacing, Å.
Barium stearate	24.4
Copper stearate	23.0 ± 4.1
Aluminum stearate	17.8 ± 4.7
Thorium stearate	20.5 ± 3.6

b. Property of Conditioned Built-up Film.—As reported in the preceding paper, copper, aluminum, and thorium ions behave in the case of adsorption on stearic acid multilayers as a fairly large unit. They also render the treated surface highly wettable as

(6) E. Kneen and W. W. Benton, *J. Phys. Chem.*, **41**, 1195 (1937).

was already reported by Inaba of our laboratory,[7] but this effect was known to be more remarkable in the case of barium stearate multilayers than in the case of stearic acid.[8] We can also observe a characteristic behavior for wetting, when we examine the built-up layers of stearate of some other heavy metal, as are summarized in Table 3.

Table 3

Wettability of Variously Conditioned Built-up Film

Conditioning		Built-up Film			
Reagent	*p*H	Barium stearate (*Y*-layer)	Copper stearate (*B*-layer)	Aluminum stearate (*B*-layer)	Thorium stearate (*B*-layer)
Barium chloride	6.5	—	—	—	—
Copper sulfate	6.5	+++	++	—	—
Aluminum chloride	6.3	++++	+++	+	—
Thorium nitrate	4.0	++++	++++	++	—

—, Non-wettable after soaking for 1 hr.; ++++, perfectly wettable after soaking within 30 sec.; +, ++, and +++, more than a half of total area of a slide being wettable after soaking for 20 min., 5 min., and 30 sec., respectively but water peeling back by the subsequent washing.

Discussion

In the data shown in Table 1, it will be noticed that the films of stearate of heavy metal are very hydrophobic, despite the difficulty of their deposition. Bikerman[9] reported that the monolayer is deposited as *Y*- or *X*-layer according to whether the contact angle being variable on both sides of 90° or variable only on the larger side of 90°. According to this view, *X*-layer should be transferred during down-trip which could be the case for copper stearate if it was built up with thorough precaution. However it was unsuccessful with aluminum and thorium stearates. This may perhaps come from their speciality in the mechanical behaviors. On the other hand, it will be noticed that the apparent spacings of all these films are smaller than that of barium stearate. Since the actual length of stearyl radical can not be considered to be affected by the presence of heavy metal, the results seem to indicate a smaller refractive index for stearate of copper, aluminum, and thorium than that of barium. The smaller refractive index in turn predicts that the stearyl radicals of these stearates exist uniformly separated in the built-up film, provided that the defection or the folding of monolayer mentioned above are absent in this case. This, together with the fact that the monolayer could hardly be transferred onto a slide on account of its mechanical resistance, leads us to the conclusion that these stearyl radicals are rigidly bound together by a somewhat long chain consisting of, say, aluminum and oxygen. Such a conclusion has been obtained also by Sasaki and Matuura[5] of our laboratory. In this connection we should refer to the macromolecular structure of aluminum soap which is recently proposed by many authors[10] to explain its peculiar and complex nature. Perhaps, we should not use the term aluminum stearate, as it was reported that we could merely find the adsorption complex consisting of hydrated alumina and stearic acid rather than the stearate.[11] But it is not a matter of fundamental importance for the present argument how they are combined together. Experimental result in Table 2 shows in agreement with these authors, that the substance thereby formed is of a complex nature.

Now, let us pay attension to the fact that the built-up films of barium, copper, aluminum, and thorium stearates exhibit nearly the same wettability towards water as shown in Table 1, which is due, of course, to the fact that a hydrocarbon part of the stearate constitutes an outermost layer in all cases. But precisely looking, we can arrange these ions in series of barium, copper, aluminum, and thorium according to the decreasing order of the wettability of their stearate, though it is not so distinct. This series is, of course, in contradiction to what would be expected from apparent spacing of stearate shown in Table 2, and can therefore be ignored as an experimental error, but we should like rather to take it for the slight indication of overturning effect allowed for molecules,[12] with the easiness decreasing in the order shown in the above series. Precisely speaking, it can reasonably be assumed that the stearate molecule is allowed to overturn itself in the surface layer, with the degree of difficulty proportional to the complexity of its structure.

A more clear evidence of this view can be

(7) A. Inaba, This Bulletin, **23**, 146 (1950).
(8) I. Langmuir and V. J. Schaefer, *J. Am. Chem. Soc.*, **59**, 1762 (1937).
(9) J. J. Bikerman, *Trans. Faraday Soc.*, **36**, 412(1940).
(10) V. R. Gray and A. E. Alexander, *J. Phys. Colloid Chem.*, **53**, 9, 23 (1949); C. G. McGee, *J. Am. Chem. Soc.*, **71**, 278 (1949); *etc.*
(11) L. J. Edwards, Thesis for Fellowship, Royal Institute of Chemistry (1946): Unfortunately the authors have not yet seen the original report.
(12) I. Langmuir, *Science*, **87**, 493 (1938).

found in Table 3. Here again it is confirmed that above ions hinder the overturning of stearic acid molecule and re-overturning of once overturned molecule, with the intensity increasing in the order mentioned above. The easiness of overturning was here corelated with the degree of wettability semiquantitatively expressed in this table. This order is also in agreement with that of the preceding report.[4] Thus we are inclined to the opinion that the metallic stearate increases the complexity of its structure in the order of barium, copper, aluminum, and thorium, among which aluminum and thorium stearates are complex enough to be called macromolecule or micelle, as was proposed by McGee *et al.*,[10] while barium stearate forms merely a simple stoichiometric compound as is usually considered. It is interesting in these relation to point out that although the film of free stearic acid or barium stearate can readily be conditioned (rendered wettable) by treating with aluminum or thorium salt solution, thorium stearate film itself is not wettable, and moreover can not be conditioned similarly, and further that stearic acid conditioned by aluminum salt proves to be fairly wettable, while aluminum stearate is well known as one of the efficient water-repellant. All these apparent contradictions can be explained reasonably. Special nature of water-repellent ferric stearate frequently employed for the coating of a trough for the surface film experiment[13] can also be understood from such a point of view.

Summary

Monolayer of stearic acid spread on water containing copper, aluminum, and thorium ions, has been transferred onto solid surface, and the apparent thickness of monolayer as well as its wettability towards water after the treatment with or without aqueous solution of salts were measured. Tentative explanations for these phenomena are given, taking into account of a macromolecular structure of aluminum soap.

The cost of this research has been defrayed from the grant of the Ministry of Education, to which the authors' thanks are due.

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(13) I. Langmuir, *Cold Spring Harbor Symposia Quant. Biol.* **6**, 171 (1938).

The Electrical Conductivity of the Condensed Polynuclear Aza-aromatic Compounds

By Hiroo Inokuchi

(Received September 18, 1951)

Introduction

The molecules of the condensed polynuclear aza-aromatic compounds are composed of the net-work plane of the conjugated double bonds of carbon and nitrogen. These substances have some interesting properties as the dyestuff material, and also as the biochemical substance. These vat dyestuffs, for example, have no "light catalytic reaction character" and can be synthesized more easily than the corresponding condensed aromatic compounds.

Little has been known about the physical properties of these compounds. For example, when the resonance energy of simple aza-aromatic compounds are compared with the corresponding hydrocarbons, the difference is negligible as shown in Table 1.[1] And it is also known that the replacement of a conjugated carbon atom by a nitrogen atom does not greatly influence the ultraviolet absorption spectrum of the compound.

Table 1

The Resonance Energy of the Condensed Aza-aromatic and Aromatic Compounds

Substance	Benzene	Pyridine	Naphthalene	Quinoline
Resonance energy, kcal./mol	41	43	77	75

(1) G. W. Wheland, "The Theory of Resonance," p. 69-70 (1944).

In the previous report,[2] we have found that violanthrone, pyranthrone etc. have the character of an intrinsic semi-conductor concerning the electrical conductivity in powdered state of these crystals. From these results, the difference in the electrical conductivity is expected to be not so large between aza-aromatic compounds and condensed aromatic hydrocarbons or quinones.

Experimental Procedure

The electrical conductivity of cyananthrone (dark violet), indanthrone black (brownish black), flavanthrone (yellowish orange), indanthrone (violet), indanthrazine (brown), 5,6-(N)-pyridino-1,9-benzanthrone (yellow) and 1,9,4,10-anthradipyrimidine (brown) was measured. The molecular structures of these compounds are illustrated in Table 3.

Indanthrone black, flavanthrone and indanthrone were purified chemically from the commercial vat dyestuffs respectively,[3] and then they were sublimed repeatedly in a vacuum until the conductivity became constant. In the case of indanthrone black (Indanthrene Black B. I. G.), as it contained a large volume of by-products, repeated chemical purifications were needed.

Indanthrazine was made by the reduction of indanthrone with E. Clar method[4] by Mr. T. Handa. And recrystallization and sublimation were repeated alternately until indanthrone was perfectly removed. In the course of this experiment, it was found that the specimen of indanthrone, for the preparation of indanthrazine, could be reduced by zinc powder more easily when it was used as a precipitated form from the concentrated sulphuric acid solution, than the ordinary product of, for example, when it was purified by the sublimation. The cause of this difference can be understood by the following explanation.

Indanthrone (Indanthrene RSN) was purified chemically and was sublimated in vacuum 10^{-4} mm.Hg at 350°. This specimen has violet colour, and is here called specimen (A). Specimen (A) was dissolved in concentrated sulphuric acid, and then this solution was poured into a large amount of water under 10° with strong stirring. This precipitated specimen was then filtered, washed to be made free from acid and dried at 70°. It is believed that no sulphate salt of indanthrone is formed in this case. This specimen has blue colour, and is called specimen (B).

When we compare x-ray Debye-Scherrer patterns of thess (A) and (B), it was found that patterns of the specimen (B) were more diffuse than the specimen (A). And in the case of the specimen (B), some of the patterns which appeared on the photograph of specimen (A), disappeared. (see Table 2.) This fact indicates that the specimen (B) is more irregular in the arrangement of molecules in crystal than (A).[5] Therefore the reactive area of the specimen (B) will be larger than specimen (A). It coinsides with the fact that the specimen (B) is reduced by zinc powder more easily than (A).

Cyananthrone was synthesized from 5,6-(N)-pyridino-1,9-benzanthrone by Mr. Handa[6] and purified in the same manner as described above.

Table 2

X-ray Data for Iadanthrone. Values of Spacings (x-ray: Cu Kα Ni-filtered)

Specimen A sublimated in vacuum		Specimen B precipitated from conc. H_2SO_4	
d/n, Å.	intensity	d/n, Å.	intensity
7.80	f	8.10	f diffuse
5.96	mf		
4.60	mf		
3.39	ff	3.46	f diffuse
2.75	mf	2.75	pp very diffuse
2.66	mp		
2.51	mp		
2.36	mf		
2.24	mf	2.24	pp very diffuse
2.12	p		
2.08	mp		
1.99	pp		
1.92	mf		
1.85	mp		
1.79	mf		
1.68	p		
1.58	mp		
1.42	p		
1.32	p		
1.275	p		
1.230	p		
1.190	pp		
1.165	pp		
1.085	pp		

As in the cases of condensed polynuclear aromatic compounds, single crystals could not be obtained. Therefore seven samples were examined in the state of the compressed powdered form. The measuring method of the electrical conductivity has been already described. The specimen was compressed between the metal end and piston in a polished good quality ebonite cylinder, which had 0.8 cm. diameter. The height of the specimen was between 0.3 cm. and 0.5 cm. The electrical resistance was measured by the potential drop

(2) H. Akamatu and H. Inokuchi, *J. Chem. Phys.*, **18**, 810 (1950); H. Inokuchi, This Bulletin, **24**, 222 (1951).
(3) H. Akamatu and K. Nagamatsu, *J. Colloid Sci.*, **2**, 503 (1947).
(4) E. Clar, *Ber.*, **72**, 1645 (1939).
(5) It was found frequently that some dyestuffs were crystallized in various ways by which the particle shape and the colour were influenced greatly (*cf.* F. A. Hamm and E. V. Norman, *J. Appl. Phys.*, **19**, 1097 (1948).
(6) T. Maki, *J. Soc. Chem. Ind. Japan*, **37**, 505 (1934).

method with the aid of a direct current amplifier using UX-54 tube. This circuit is illustrated in Fig. 1. The supplied voltage was 400~500 V. per cm. The constancy of the electrical conductivity of these powdered specimens is obtained when the compressed pressure upon them is over 80 kg./cm.^{2} Therefore these specimens were compressed by a lever above 80 kg./cm.^{2}

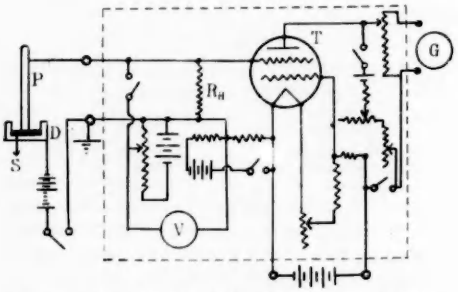


Fig. 1.—The circuit of direct current amplifier: T, electrometer tube UX-54 (This tube has the same character as FP-54); V, voltmeter. (Range is from 0.01 V. to 5 V.); R_H, high resistance ($10^9\Omega$ or $10^{10}\Omega$); G, galvanometer, (Sensitivity is 0.001 μA./mm.); P, piston (0.8 cm. diameter); D, Cyrindrical dish (made of brass coated by chromium); S, specimen. The height of this is between 0.1 cm. and 0.5 cm.

On the other hand, as 5, 6-(N)-pyridino-1, 9-benzanthrone (No. 6) and 1, 9, 4, 10-anthradipyrimidine (No. 7) have high electrical resistivity, these specimens(S)were compressed on a cylindrical dish (D), instead of the ebonite cylinder, by a piston (P) to 80 kg./cm.^{2} with a lever system as shown in Fig. 1 schematically. Because the ebonite cylinder was not used, the surface leakage of the cylinder could be avoided completely and the temperature could be increased to 300°. By this method we could measure the high electrical resistance of these substances.

Results and Discussions

The temperature coefficient of the electrical resistivity was negative in every specimen, and logarithm of electrical resistivity $\log \rho$ and the reciprocal of temperature $1/T$ have the linear relation for a large range of temperature,

$$\rho = \rho_0 \exp(\Delta\varepsilon/2kT) \qquad (1)$$

where $\Delta\varepsilon$ is the activation energy, corresponding to the interval of energy band on the model of semi-conductor.

About these behaviours, differences could not be found between this group and condensed polynuclear aromatic compounds.

Non-hysteresis of electrical resistivity and independency of the current upon the time prove the electronic conductance. Therefore the mechanism of semi-conductive character of those aza-aromatic compounds is assumed to be intrinsic, from their perfect molecular crystals as in the cases of the condensed polynuclear aromatic compounds.

The value of ρ_{15}, extrapolated to 15°, $\Delta\varepsilon$ and ρ_0 were tablulated in Table 3, and the relation of equation (1) were illustrated on Figs. 2 a, b and c.

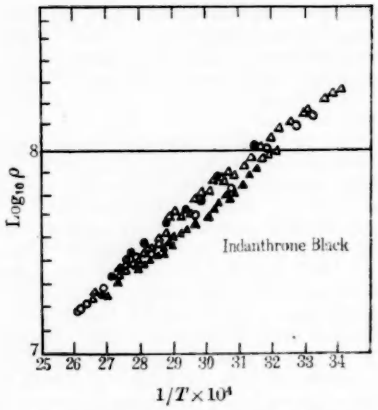


Fig. 2 a.—The relation between resistivity and temperature for indanthrone black: △82.1 kg./cm.^{2}, increasing temperature; ▲82.1 kg./cm.^{2}, decreasing temperature; ○119.5 kg./cm.^{2}, increasing temperature; ●119.5 kg./cm.^{2}, decreasing temperature.

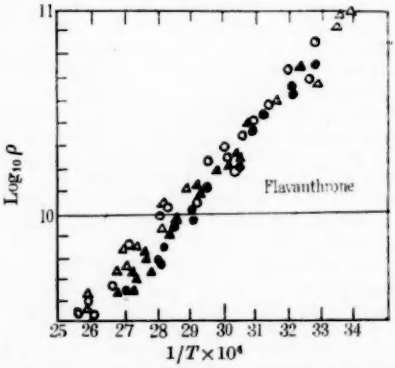
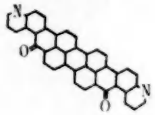
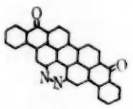
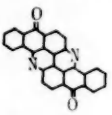
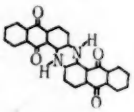
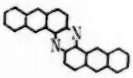


Fig. 2 b.—The relation between resistivity and temperature for flavanthrone. The same symbols as in Fig. 2 a.

Concerning the value of the electrical conductivity, cyananthrone and indanthrone black

Table 3

The Values of ρ_{15}, $\Delta\varepsilon$ and ρ_0 of the Condensed Polynuclear Aza-aromatic Compounds

No.	Substance	Structure	ρ_{15}, Ω cm.	$\Delta\varepsilon$, e. V.	ρ_0, Ω cm.
1	Cyananthrone		1.2×10^{7}	0.20	2.24×10^{3}
2	Indanthrone Black		2.5×10^{8}	0.56	3.45×10^{3}
3	Flavanthrone		1.4×10^{11}	0.70	9.5×10^{4}
4	Indanthrone		7.5×10^{14}	0.63	2.5×10^{9}
5	Indanthrazine		1.4×10^{15}	0.66	2.2×10^{9}
6	5, 6-(N)-Pyridino-1, 9-benzanthrone		8.5×10^{22}	3.20	1.4×10^{-5}
7	1, 9, 4, 10-Anthradipyrimidine		8.8×10^{24}	3.21	1.3×10^{-7}

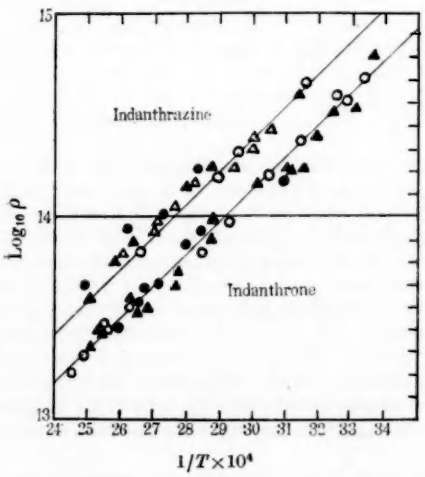


Fig. 2c.—The relation between resistivity and temperature for indanthrone and indanthrazine. The same symbols as in Fig. 2a.

have the typical values of semi-conductor, $10^{-7}\Omega^{-1}$cm.$^{-1}$~$10^{-8}\Omega$cm.$^{-1}$[7]

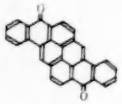
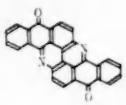
When the number of rings is increased, the illustration shows that the value of electrical conductivity and activation energy approach those of carbon. The electrical conductivity and the activation energy of commercial graphite is the order of $10^{2}\,\Omega^{-1}$ cm.$^{-1}$ and 10^{-2} e. V. respectively[8].

It is found that the values of the electrical resistivity of aza-aromatic compounds are much smaller than those of related aromatic compounds. A typical one is shown in Table 4. It is interesting to compare this result to the fact that intensities of flourescence spectra of aza-aromatic compounds (6-azachrysene and 1-azapyrene) are about ten times as great as those of the related hydrocarbons (chrysene and pyrene)[9].

(7) M. F. Manning and M. E. Bell, *Rev. Mod. Phys.*, **12**, 215 (1940).
(8) S. Mrozowski, *Phys. Rev.*, **77**, 838 (1950).
(9) R. Schoental and E. J. Y. Scott, *J. Chem. Soc.*, 1283 (1949).

Table 4

The Values of $\Delta\varepsilon$ and ρ_{15} of Pyranthrone and Flavanthrone

Substance	Pyranthrone 	Fravanthrone 
$\Delta\varepsilon$	1.06 e.V.	0.70 e.V.
ρ_{15}	$3.9\times10^{15}\Omega$ cm.	$1.4\times10^{11}\Omega$ cm.

The electrical conductivity of indanthrone is very poor as compared with the value of flavanthrone. We think that the cause of this irregularity is a spatial structure. The spatial structure of indanthrone has not yet been researched, but a magnetic susceptibility of this suggests non-coplaner structure.(10)

Summary

The electrical conductivity of the condensed polynuclear aza-aromatic compounds; (1) cyananthrone, (2) indanthrone black, (3) flavanthrone, (4) indanthrone, (5) indanthrazine, (6) 5, 6-(N)-pyridino-1, 9-benzanthrone and (7) 1, 9, 4, 10-anthradipyrimidine,—has the character of a semi-conductor. The electrical resistivity of these compounds can be expressed as $\rho=\rho_0\exp(\Delta\varepsilon/2kT)$ and the mechanism of the conduction is assumed to be that of the intrinsic semi-conductor as in the cases of the condensed polynuclear aromatic compounds. The value of $\Delta\varepsilon$ and ρ at 15° were found as; (1) 0.20 e.V., $1.2\times10^{7}\Omega$ cm., (2) 0.56 e.V., $2.5\times10^{5}\Omega$ cm., (3) 0.70 e.V., $1.4\times10^{11}\Omega$ cm., (4) 0.63 e.V., $7.5\times10^{14}\Omega$ cm., (5) 0.66 e.V., $1.4\times10^{15}\Omega$ cm., (6) 3.2 e.V., $8.5\times10^{22}\Omega$ cm. and (7) 3.21 e.V., $8.8\times10^{24}\Omega$ cm. The values of the electrical resistivity of this group are smaller than that of related condensed polynuclear aromatic compounds.

The author expresses his hearty thanks to Prof. H. Akamatu for his kind direction of this study and also to Mr. T. Handa who prepared the samples of this work. The Debye-Sherrer x-ray photograph was due to Mr. H. Takahashi's favour. The author wishes to thank him. The cost of this research has been defrayed from the Grant in Aid for Fundamental Scientific Research from the Ministry of Education, to which the author's thanks are due.

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(10) H. Akamatu, suggestion.

On the Viscosity of Binary Mixture of Liquids(1)

By Mikio TAMURA and Michio KURATA

(Received September 20, 1951)

Introduction

If we attempt to derive an equation which should connect the viscosity coefficient of a mixture of liquids A and B with its composition and those of its constituents, it becomes necessary, in addition, to introduce a quantity in order to take the molecular interaction of A-B type into account inasmuch as the viscosity of a mixture depends upon the mutual action of the molecules constituting it. Dolezalek,(2) at first, and then Wijk(3) introduced the quantity relating the A-B type interaction in the form of a parameter called "mutual viscosity" η_{AB} and derived their own equations having simple form, but the agreement of their equations with the experimental values seems not to be enough as will be shown below, and their definitions of η_{AB} seem to be obscure. Recently, Powell, Roseveare and Eyring(4) and Kottler(5) proposed the semi-empirical equations respectively, which involve the quantities of different type from η_{AB}, but their equations are inconvenient for numerical

(1) The contents of this paper were read at the 1st and 2nd annual meetings of the Society in May, 1948 and in April, 1949 and were published partly in *Busseiron Kenkyu*, **11**, 20 (1948); **20**, 151 (1949) in Japanese.

(2) F. Dolezalek, *Z. phys. Chem.*, **83**, 73 (1913).

(3) A. J. A. van der Wijk, *Nature*, **138**, 845 (1936).

(4) R. E. Powell, E. Roseveare and H. Eyring, *Ind. Eng. Chem.*, **33**, 430 (1941).

(5) F. Kottler, *J. Phys. Chem.*, **47**, 277 (1943); **48**, 76 (1944).

calculation, and this is an important defect for practical use. In this paper, the authors will give the definition of η_{AB} explicitly and derive a new semi-empirical equation which has simple form and seems to be better than others.

Derivation of Equation

According to Frenkel and Eyring,[6] the viscosity coefficients, η_A and η_B, of liquids A and B are given by

$$\eta_A=(kT/v_A)\tau_A \quad \text{and} \quad \eta_B=(kT/v_B)\tau_B, \tag{1}$$

respectively, where k is the Boltzmann constant, T the absolute temperature and v's are the volumes occupied by one molecule; the subscripts indicate the molecular species. And τ's represent the relaxation times of flow and are given by

$$\left.\begin{aligned}\tau_A&=(h/kT)\exp(\chi_A/kT)\\ \text{and}\quad \tau_B&=(h/kT)\exp(\chi_B/kT),\end{aligned}\right\} \tag{2}$$

where h is the Planck constant and $\chi_A(\chi_B)$ is the activation free energy of flow relating only with A-A(B-B) contact of molecules. Quite similarly, the viscosity coefficient η of mixture of liquids A and B, should be given by

$$\eta=(kT/v)\tau \tag{3}$$

$$\text{with} \qquad \tau=(h/kT)\exp(\chi/kT). \tag{4}$$

Here the letters with no subscript (τ, v and χ etc.) represent the quantities regarding the mixture.

Now, let us introduce the "mutual activation free energy of flow" χ_{AB} relating only with A-B contact of molecules in the same manner as χ_A (or χ_B) relates with A-A (or B-B) contact, and define τ_{AB} by

$$\tau_{AB}=(h/kT)\exp(\chi_{AB}/kT). \tag{5}$$

This is the relaxation time of flow of A molecule in liquid B or *vice versa*. And τ in Eq. (3) may be given as a kind of mean of τ_A, τ_B and τ_{AB}.

If we represent the molar fractions of each component by x_A and x_B, the probabilities of occurring of A-A, B-B and A-B contacts of molecules are given by x_A^2, x_B^2 and $2x_Ax_B$ respectively under the condition of random mixing. Then assuming that τ is given by the arithmetical mean of τ's, *i. e.*,

$$\tau=x_A^2\tau_A+x_B^2\tau_B+2x_Ax_B\tau_{AB}, \tag{6}$$

and defining the "mutual viscosity coefficient" η_{AB} by

$$\eta_{AB}=\{kT/(v_Av_B)^{1/2}\}\tau_{AB}, \tag{7}$$

we obtain

$$\eta=x_A^2\left(\frac{v_A}{v}\right)\eta_A+x_B^2\left(\frac{v_B}{v}\right)\eta_B+2x_Ax_B\left(\frac{v_Av_B}{v^2}\right)^{1/2}\eta_{AB}, \tag{8}$$

from Eqs. (1), (3), (6) and (7). If d be the density and w the weight fraction, we can obtain easily

$$x_Av_A/v=w_Ad/d_A \text{ and } x_Bv_B/v=w_Bd/d_B. \tag{9}$$

For the practical purposes, we can put

$$x_Av_A/v=\varphi_A \quad \text{and} \quad x_Bv_B/v=\varphi_B, \tag{10}$$

where φ's are the volume fractions, and obtain

$$\eta=x_A\varphi_A\eta_A+x_B\varphi_B\eta_B+2(x_Ax_B\varphi_A\varphi_B)^{1/2}\eta_{AB}, \tag{11}$$

since the volume change on mixing can be neglected in general. Although Eq. (11) appears to be rather similar to the Dolezalek equation,

$$\eta=x_A^2\eta_A+x_B^2\eta_B+2x_Ax_B\eta_{AB}, \tag{12}$$

it must be noticed that the agreement of the former with the experiments is considerably better than that of the latter as will be shown in the following section.

It is well-known that, if we use the harmonic oscillator model of liquids,[7] we can get the relations of the following type;

$$\left.\begin{aligned}&\tau=(1/\nu)\exp(\varepsilon/kT),\\ &\tau_A=(1/\nu_A)\exp(\varepsilon_A/kT)\\ \text{and}\quad &\tau_{AB}=(1/\nu_{AB}\nu_{BA})^{1/2}\exp(\varepsilon_{AB}/kT),\\ &\text{etc.,}\end{aligned}\right\} \tag{13}$$

where ε's are the activation energies of flow and ν's are the frequencies of lattice vibration and ν_{AB} (or ν_{BA}) represents the frequency of A (or B) molecule surrounded by B (or A) molecules only. Then (1), (3) and (7) with (13) become

(6) J. Frenkel, "Kinetic Theory of Liquids," Oxford, 1947, Chap IV, § 2; S. Glasstone, K. J. Laidler and H. Eyring, "The Theory of Rate Processes," New York, 1941, Chap. IX.

(7) See, for example, R. H. Fowler and E. A. Guggenheim, "Statistical Thermodynamics," Cambridge, 1939, p. 319.

$$\left.\begin{aligned} &\eta=(kT/v\nu)\exp(\varepsilon/kT),\\ &\eta_A=(kT/v_A\nu_A)\exp(\varepsilon_A/kT)\\ \text{and}\quad &\eta_{AB}=\{kT/(v_Av_B\nu_{AB}\nu_{BA})^{1/2}\}\\ &\qquad\times\exp(\varepsilon_{AB}/kT). \end{aligned}\right\}\quad(14)$$

That is, the temperature dependency of η_{AB} may also satisfy the expression of Andrade's type.[8]

Comparison of η with the Experimental Values

The comparison of Eq. (11) with the experimental values of viscosity coefficient, η_{obs}, has been performed in regard to about 30 systems which are chosen from various types of mixtures as shown in Tables 1 and 2. In these tables are arranged the systems in three classes; mixtures of (A) non-polar and non-polar liquids, (B) non-polar and polar liquids and (C) polar and polar liquids, for convenience' sake. And Δ denotes the arithmetical mean of the absolute values of $\Delta=(\eta_{calc}-\eta_{obs})\times 100/\eta_{obs}$ at various compositions and max. or min. in the last column of these tables indicate that $\eta-x$ curve has a maximum or minimum. The unit of η_{AB} is "centi-poise" and the values of η of all systems except B-2, 4 and C-7 are adopted from "International Critical Tables," Vol. V. Some of the results of calculation are plotted in Figures 1~3, where the circles (○ or ⊕) and the full lines represent the observed

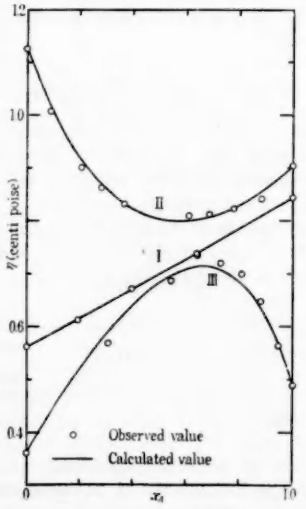

Fig. 1.—Observed and calculated values of η —1: Curve I, A=CCl_4, B=C_6H_6(30°C.); Curve II, A=CCl_4, B=CH_3COOH(25°C.); Curve III, A=H_2O, B=CH_3OH(60°C.).

(8) E. N. da C. Andrade, *Phil. Mag.*, **17**, 497, 698 (1934).

Table 1

List of the Systems Compared and the Results Obtained.—I

No.	System (Observer)	°C.	η_{AB}	$\bar{\Delta}$, %	
A-1	$CCl_4+C_2Cl_4$ (i)	25	0.752	0.5	min.
A-2	$CCl_4+C_2H_2Cl_4$ (i)	25	0.973	0.9	
A-3	$CCl_4+C_6H_6$ (ii)	0	1.134	0.0	
		10	0.941	0.0	
		20	0.797	0.2	
		30	0.688	0.2	
		40	0.596	0.1	
		50	0.522	0.0	
		60	0.463	0.2	
A-4	$CS_2+C_6H_6$ (iii)	25	0.474	0.6	
B-1	CCl_4+CHCl_3 (iii)	25	0.589	0.7	
B-2	CCl_4+CH_3COOH(xii)	25	0.657	0.9	min.
B-3	$CCl_4+CH_3COCH_3$(iv)	0	0.538	2.8	
B-4	CCl_4+n-$C_6H_{17}OH$ (xii)	25	1.112	3.7	
B-5	$CS_2+CH_3COCH_3$(v)	−13	0.450	0.2	min.
		−10	0.445	0.2	min.
		0	0.416	0.4	min.
		15	0.389	0.0	
		35	0.326	0.2	
B-6	$CS_2+C_2H_5OH$ (vi)	25	0.510	2.9	
B-7	$CS_2+C_6H_5CH_3$ (iii)	25	0.442	0.9	
B-8	$C_2H_2Cl_4+C_6H_5NO_2$ (vii)	5	3.41	1.4	max.
		76.5	0.958	0.7	max.
B-9	$C_6H_6+CHCl_3$ (iii)	25	0.572	0.2	
B-10	$C_6H_6+CH_3OH$ (viii)	25	0.636	0.2	
B-11	$C_6H_6+CH_3COOH$(vi)	25	0.464	2.9	min.
B-12	$C_6H_6+C_2H_5OH$ (vi)	25	0.524	1.8	min.
	〃 (ix)	15	0.668	1.9	
		20	0.614	2.5	min.
		25	0.567	2.0	min.
		30	0.522	1.4	min.
		35	0.481	0.9	min.
		40	0.445	0.9	
B-13	$C_6H_6+C_6H_5OH$ (x)	20	—	—	
B-14	$C_6H_6+C_6H_5CH_2CO_2C_6H_5$ (xi)	25	—	—	
		40	0.905	6.6	
		60	0.751	2.5	
		75	0.660	1.2	

Observer of η: (i) Herz and Rathmann, (ii) Thorpe and Rodger, (iii) Linebarger, (iv) Sakhanov and Ryakhovskii, (v) Faust, (vi) Dunstan, (vii) Drucker and Kassel, (viii) Bramley, (ix) Gertman, (x) Oholm, (xi) Bingham and Sarver, (xii) Jones, Bowden, Yarnold and Jones, *J. Phys. Coll. Chem.*, **52**, 753 (1948).

and the calculated values respectively. For purposes of comparison, we show also in Fig. 3 the values obtained from the Dolezalek equation, (12), and the Wijk equation,[3]

$$\log \eta = x_A^2 \log \eta_A + x_B^2 \log \eta_B + 2x_A x_B \log \eta_{AB}. \quad (15)$$

Table 2

List of the Systems Compared and the Results Obtained.—II

No.	System (Observer)	°C.	η_{AB}	Δ, %	
C-1	H_2O+CH_3OH (i)	0~30	—	—	
		40	1.510	5.1	max.
		50	1.232	4.2	max.
		60	0.997	2.1	max.
C-2	$H_2O+C_2H_5OH$ (ii)	0~40	—	—	
		45	1.940	7.0	
		50	1.721	6.3	
		55	1.532	5.6	
		60	1.370	5.1	max.
		65	1.230	4.7	
		70	1.108	4.5	
		75	1.000	4.3	
C-3	$H_2O+C_3H_7OH$ (iii)	20	5.31	6.5	
		25	4.50	6.0	max.
		30	3.82	5.2	max.
C-4	$H_2O+C_3H_5(OH)_3$		—	—	
C-5	$CHCl_3+C_2H_5OC_2H_5$ (iv)	0	0.626	3.8	
		10	0.543	3.4	
		20	0.474	2.9	
		30	0.418	2.5	
C-6	$CHCl_2F+CH_3COCH_3$ (v)	0	0.562	1.2	max.
C-7	$CH_3OH+C_2H_5OH$ (vi)	25	0.730	0.6	
		35	0.626	0.5	
		45	0.546	0.2	
		55	0.484	0.4	
C-8	$C_2H_5OC_2H_5+C_6H_5OC_6H_5$ (vii)		—	—	
C-9	i-$C_5H_{11}OH+HCONH_2$ (viii)	0	13.20	7.6	max.
		76.5	1.560	2.3	max.
C-10	i-$C_5H_{11}OH+C_6H_5NO_2$ (viii)	0	1.650	6.4	min.
		80	0.503	1.2	min.
C-11	i-$C_5H_{11}OH+C_6H_5N(C_2H_5)_2$ (viii)	0	2.77	6.1	min.
		76.5	0.559	1.2	min.
C-12	$C_6H_5CH_3+C_6H_5CO_2C_2H_5$ (ix)	25	0.782	1.2	

Observer of η: (i) Traube, (ii) Bingham and Jackson, (iii) Dunstan and Thole, (iv) Thorpe and Rodger, (v) Lacher, Walden and Park, *J. Am. Chem. Soc.*, **71**, 3026 (1949), (vi) Bingham, White, Thomas and Cadwell, (vii) Kendall and Wright, (viii) Drucker and Kassel, (ix) Kendall and Monroe.

It is nothing but the illustration of the satisfactory agreement of our equation (11) with the experimental values, that $\bar{\Delta}$ of almost all the systems is less than a small percentage. It must be noticed that Δ of the mixture

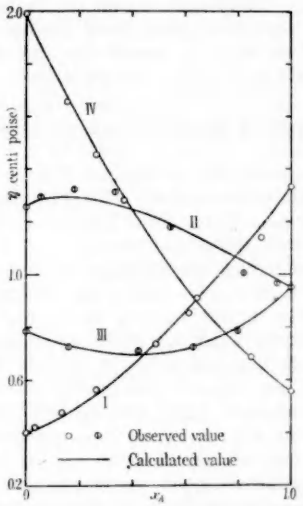

Fig. 2.—Observed and calculated values of η —2: Curve I, A=CCl_4, B=CH_3COCH_3 (0°C.); Curve II, A=i-$C_5H_{11}OH$, B=$HCONH_2$ (76.5°C.); Curve III, A=i-$C_5H_{11}OH$, B=$C_6H_5N(C_2H_5)_2$ (76.5°C.); Curve IV, A=$C_6H_5CH_3$, B=$C_6H_5CO_2C_2H_5$ (25°C.).

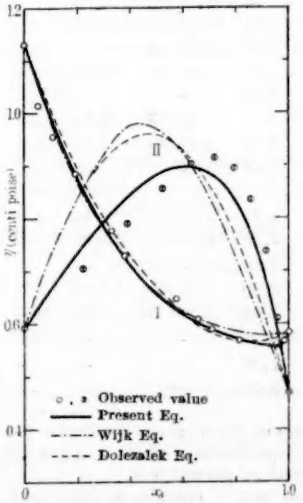

Fig. 3.—Observed and calculated values of η —3: Curve I, A=C_6H_6, B=C_2H_5OH(25°C.); Curve II, A=H_2O, B=C_2H_5OH(60°C.).

composed of liquids strongly associated becomes also less than a small percentage at a higher temperature (*cf.* Systs. C-1, 2 and 9∼11). Consequently, the equation presented here seems to be better than the equation of either Dolezalek or of Wijk. However, our equation, as well as others, seems not to be applicable to the mixture in which the difference between η_A and η_B is very remarkable (for example, the mixture of water and glycerine).

Mutual Activation Energy of Flow

If we plot $\log \eta_{AB}$ against $1/T$, a straight line will be obtained according to the definition of η_{AB}, (7) or (14). The demand for this linearity is, indeed, satisfied by our results of calculation and, accordingly, we can estimate the value of ε_{AB} from the slope of this straight line. The values of ε_{AB} thus obtained are tabulated in Table 3. As is expected, ε_{AB} is approximately equal to $(\varepsilon_A+\varepsilon_B)/2$ in such systems as carbon tetrachloride+benzene or methyl alcohol+ethyl alcohol which are regarded as the so-called "ideal solutions." But in the mixture of water and alcohols, ε_{AB} is remarkably larger than ε_A or ε_B; ε of pure components of these systems being 3.30 kcal./mole for water, 2.51 for methyl alcohol and 3.52 for ethyl alcohol at about 60°C. This result may be related closely with the strong affinity of alcohols with water and with the remarkable volume contraction which results on mixing. In Table 3, the values of χ_{AB} calculated from (7) with (5) or

$$\chi_{AB}=kT\ln\{(v_Av_B)^{1/2}\eta_{AB}/h\} \qquad (16)$$

are also tabulated.

Table 3

Mutual Activation Energy of Flow.

Syst. No.*	ε_{AB}†	χ_{AB}(°C.)**	λ_{AB}	$\lambda_{AB}/\varepsilon_{AB}$	λ_{AB}/χ_{AB}
A-3	2.69	3.13(60)	7.75	2.9	2.48
4	—	2.66(25)	6.50	—	2.44
B-5	1.19	2.47(35)	6.62	5.6	2.68
7	—	2.67(25)	6.70	—	2.51
8	3.46	—	—	—	—
9	—	2.86(25)	7.75	—	2.71
12	2.90	2.75(40)	8.73	3.0	3.12‡
14	1.98	—	—	—	—
C-1	4.52	2.82(60)	9.81	2.2	3.48‡
2	4.88	3.08(75)	10.22	2.1	3.32‡
3	5.18	—	—	—	—
5	2.20	2.76(30)	7.00	3.2	2.54
7	2.65	2.99(55)	9.50	3.6	3.51‡
9	5.31	—	—	—	—
10	2.86	—	—	—	—
11	3.98	—	—	—	—

* System No. is given in Tables 1 and 2.
** Temperatures at which χ_{AB} was calculated are put in parentheses.
† The unit of energies is kcal./mole.
‡ Compare with the values; $\lambda/\chi=4.98(H_2O)$, $3.78(CH_3OH)$ and $3.34(C_2H_5OH)$ at about 60°C.

Further, the empirical relations of ε and χ of pure liquid with its heat of vaporization, λ, proposed by Eyring seem to hold good for ε_{AB} and χ_{AB}, too, as shown in the table: that is,

$$\lambda_{AB}/\varepsilon_{AB}=3\sim4 \quad \text{and} \quad \lambda_{AB}/\chi_{AB}\fallingdotseq2.5, \qquad (17)$$

where λ_{AB} denote the "mutual heat of vaporization" and it can be obtained from the equations of vapor pressure P of binary liquid mixture,

$$P=x_A\varphi_AP_A+x_B\varphi_BP_B+2(x_Ax_B\varphi_A\varphi_B)^{1/2}P_{AB} \qquad (18)$$

with $P_{AB}=\text{constant}\times\exp(-\lambda_{AB}/kT)$.

In addition, the linearity of the $\log\eta_{AB}$ against $1/T$ is very useful for practical purposes, because we can calculate η_{AB} at any temperature so far as the values are known, at least, at two temperatures.

Activation Energy of Flow of Mixture

We can obtain the activation energy of flow of mixture ε from η_{obs} by

$$\varepsilon_{obs}=\left\{\frac{\partial\ln(\eta_{obs}v/T)}{\partial(1/kT)}\right\}_{x_A}. \qquad (19)$$

On the other hand, substituting (11) for (19) and performing the differentiation, we obtain the following expression for ε after some rewritings;

$$\varepsilon_{calc}=y_{AA}\varepsilon_A+y_{BB}\varepsilon_B+y_{AB}\varepsilon_{AB}, \qquad (20a)$$

with $y_{AA}=x_A\varphi_A\eta_A/\eta_{calc}$, $y_{BB}=x_B\varphi_B\eta_B/\eta_{calc}$ and $y_{AB}=2(x_Ax_B\varphi_A\varphi_B)^{1/2}\eta_{AB}/\eta_{calc}$. (20b)

Here y's are regarded as the occurring probabilities of molecular contacts of the indicated

(9) Eq. (18) will be published in future. As will be shown in our later paper, the value of λ_{AB} obtained from Eq. (18) agrees with the one estimated from the heat of mixing.

types. The comparison of ε_{calc} with ε_{obs} is given in Fig. 4. Here the mixture of carbon tetrachloride and benzene and that of water and ethyl alcohol are chosen as the examples of the ideal cases and non-ideal respectively. In this figure, the values calculated from the Wijk equation, (15) or

$$\varepsilon = x_A^2\varepsilon_A + x_B^2\varepsilon_B + 2x_Ax_B\varepsilon_{AB}, \qquad (21)$$

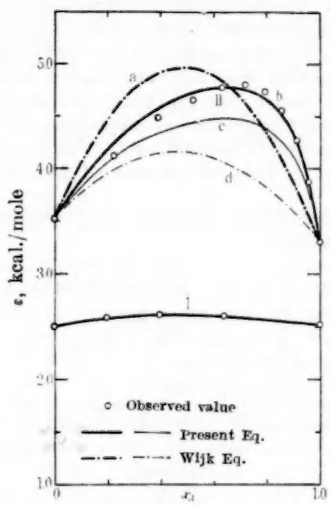

Fig. 4.—Observed and calculated values of ε: Curve I, A=CCl_4, B=C_6H_6, ε_{AB}=2.69 kcal./mole; Curve II, A=H_2O, B=C_2H_5OH (at about 60°C.), ε_{AB}=, (a), 6.50 (b), 5.30 (c and d) 4.88 kcal./mole.

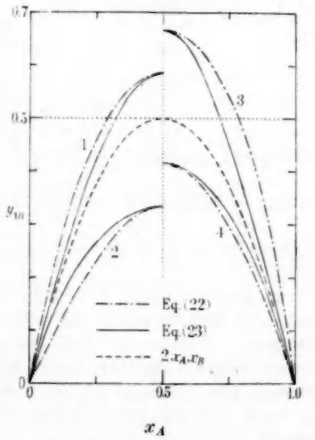

Fig. 5.—Occurring probabilities of A-B contact, y_{AB}: $\tau_A/\tau_{AB}=\tau_B/\tau_{AB}=1/\sqrt{2}$ (curve 1); 2 (curve 2); 1/2 (curve 3); $\sqrt{2}$ (curve 4). Eq. (23) nearly coincides with the Bethe-Fowler-Takagi approximation.

are also plotted for comparison's sake. Though in the former example, either (20) or (21) agree with ε_{obs} satisfactorily. In the latter, (21) used by Wijk does not agree with ε_{obs} even if the values of ε_{AB} were adjusted in any way. On the contrary, (20) agrees with ε_{obs} satisfactorily, if we adjust ε_{AB} so that ε_{calc} may agree with ε_{obs}. Though the values of ε_{AB} thus determined, 5.30 kcal./mole, is slightly different from that of ε_{AB} in Table 3, 4.88 kcal./mole, the inconsistency in this degree will be acceptable in such non-ideal systems as this example.

Now, let us examine the physical meaning of the assumption (6) used in order to derive Eq. (11). For that, it seems to be simplest to examine the meaning of y's which have been given by (20b). Substituting (1), (3), (6) and (7) into (20b), we obtain

$$\left.\begin{aligned} y_{AA} &= x_A^2\tau_A/(x_A^2\tau_A + x_B^2\tau_B \\ &\quad + 2x_Ax_B\tau_{AB}) = x_A^2\tau_A/\tau, \\ y_{BB} &= x_B^2\tau_B/\tau \end{aligned}\right\} \qquad (22)$$

and $y_{AB} = 2x_Ax_B\tau_{AB}/\tau$.

If the difference between τ_A and τ_B be not so remarkable, we can transform (22) to the following forms after some tolerable approximations:[10]

$$y_{AB} = 4x_Ax_B/(1+\beta) \quad \text{etc.}, \qquad (23a)$$

with $\beta \fallingdotseq [1+4x_Ax_B\{(\tau_A\tau_B/\tau_{AB}^2)-1\}]^{1/2}$. (23b)

These equations are to be compared with those of the Bethe-Fowler-Takagi (B-F-T) approximation for regular solutions.[11] That is, according to this approximation y's are, likewise, given by (23a) with

$$\beta = [1+4x_Ax_B\{\exp(\Delta\zeta/kT)-1\}]^{1/2}, \qquad (24)$$

where $\Delta\zeta$ denotes the increase of the free energy in the process,

$$\text{(A-A contact)} + \text{(B-B contact)} \rightarrow 2\times\text{(A-B contact)}.$$

Though (23b) and (24) seem to be slightly different, both expressions of β agree with

(10) Since the agreement of (22) with (23) is satisfactory as will be shown in Fig. 5, the rewriting from (22) to (23) seems to be reasonable.

(11) Ref. 7, p. 358; Y. Takagi, *Proc. Phys. Math. Soc. Japan*, **23**, 44 (1941).

(12) The free energies, ζ's of molecular contacts of the indicated types are given by

$\zeta_{AA} = (2\lambda_A/z) - (6kT/z)\ln\nu_A + (3kT/z)\ln(kT/2\pi m_A)$ etc., so far as the harmonic oscillator model of liquids is used (See, Ref. 7, p. 324). Since the last terms in the right hand side of these formulae are canceled in $\Delta\zeta = \zeta_{AA} + \zeta_{BB} - 2\zeta_{AB}$, we can obtain Eq. (25).

each other as shown in the followings. Using the harmonic oscillator model of liquids, we can put[2]

$$\exp\left(\frac{\Delta\zeta}{kT}\right)=\left(\frac{\nu_{AB}\nu_{BA}}{\nu_A\nu_B}\right)^{6/z}\exp\left\{\frac{\lambda_A+\lambda_B-2\lambda_{AB}\left(\frac{2}{z}\right)}{kT}\right\}, \quad (25)$$

where z is the number of the nearest neighbors. Then, using $z\fallingdotseq 8$, the result obtained by the x-ray diffraction, and the Eyring relation (17), we obtain

$$\exp\left(\frac{\Delta\zeta}{kT}\right)\fallingdotseq\left(\frac{\nu_{AB}\nu_{BA}}{\nu_A\nu_B}\right)^{3/4}\exp\left\{\frac{\varepsilon_1+\varepsilon_B-2\varepsilon_{AB}}{kT}\right\}\fallingdotseq\frac{\tau_A\tau_B}{\tau_{AB}^2}, \quad (26)$$

from (25) and (13), for $\nu_{AB}\nu_{BA}/\nu_A\nu_B\fallingdotseq 1$ in general. Accordingly, it can be concluded that (23), nearly, coincides with the B-F-T approximation. In Fig. 5, we show the values of y_{AB} calculated from (22) and (23) and the value of $2x_Ax_B$ for some values of τ's. In conclusion, our treatment involving (22) corresponds to the B-F-T approximation. From the fact mentioned here, the reason becomes obvious why Eq. (11) corresponding to (22) is better, as shown in before, than the Wijk equation corresponding to (21) or to the Bragg-Williams approximation. Furthermore, it will be one of the strong points of (22) in comparison with (21) that the effect of the difference between the sizes of constituent molecules is tacitly taken into consideration by the weight factor, τ's, having the values which are parallel with the molecular sizes in general.

Summary

A new semi-empirical equation for the viscosity coefficient of binary mixture of liquids, Eq. (11), has been derived and we have reached the conclusion that the assumption used in order to derive our equation corresponds to the Bethe-Fowler-Takagi approximation for regular assembly qualitatively. The equations for η and ε have been compared with the experimental values and satisfactory results have been obtained.

In conclusion, the authors would like to express their thanks to Prof. T. Watari and Mr. K. Maeda for some helpful discussions. This work was aided by a grant from the Ministry of Education.

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Note on the Ideal Mixture Law for Viscosity

By Tetsuya ISHIKAWA

(Received September 26, 1951)

Four interesting measurements on the viscosity of mixtures of the same crystal forms were seen in the recent literature, three[1] being those of ionic liquids with tests of Macleod's formula (1924), and one[2] that of the sodium and potassium system with test of Drucker-Kassel's formula (1911). According to their results these formulas hold good with at most 2 % and 1 % divergence respectively. Among the known formulas, however, there are two more worthy to be considered: the one is Kendall's formula (1913)[3]

$$\log\eta=(1-z_m)\log\eta_1+z_m\log\eta_2$$

and the other is Ishikawa's formula (1929)[4]

$$\eta=\eta_1+(\eta_2-\eta_1)Kz_m/(1-z_m+Kz_m)$$

where η_1, η_2 and η denote the viscosities of component 1, 2 and mixture respectively, z_m molar fraction of component 2, K the characteristic constant which signifies, according to the latter's theory, the intensity of the molecular attraction field of component 2 relative to that of component 1.

Tests made with these four pairs are illustrated in tables, from Table 1 to Table 4, in which

(1) B. S. Harrap and E. Heymann, *Chem. Rev.*, **48**, 45 (1951).

(2) C. T. Ewing, J. A. Grand and R. R. Miller, *J. Am. Chem. Soc.*, **73**, 1168 (1951).

(3) J. Kendall, *Meddelanden f. K. Velensk, Novelinstitute*, **2**, No. 25, 1 (1913).

(4) T. Ishikawa, This Bulletin, **4**, 5 (1929).

only percentage deviations $100(\eta_{calc}-\eta_{obs})/\eta_{obs}$ are given for comparison of those obtained by the respective observers.

Table 1

AgBr (1) – AgCl (2), 500°C.[3]

z_m	Deviation, %		
	Macleod	Kendall	Ishikawa (K=1.21)
0.200	+0.5	+0.5	0
0.400	+0.5	0	0
0.599	+1	+0.5	0
0.732	−2	−0.5	−0.5

Table 2

$CdCl_2$ (1) – $CdBr_2$ (2), 600°C.[3]

z_m	Deviation, %		
	Macleod	Kendall	Ishikawa (K=0.82)
0.252	0	−0.5	−0.5
0.445	+1	+0.5	0
0.725	+0.5	+0.5	0

Table 3

$PbBr_2$ (1) – $PbCl_2$ (2), 510°C.[3]

z_m	Deviation, %		
	Macleod	Kendall	Ishikawa (K=0.33)
0.247	0	+3.5	−1
0.436	+1	+7.5	+0.5
0.769	−2	+9	+2

Table 4

Na (1) – K (2).[2]

z_m	°C.	Deviation, %		
		Drucker-Kassel	Kendall	Ishikawa (K=2.54)
0.214	103.7±0.2	−0.9	+6.6	−0.1
	121.5±0.2	−1.1	+6.1	−0.5
	147.0±0.2	−0.9	+6.0	−0.5
	167.4±0.2	−0.7	+5.9	−0.4
	192.8±0.2	−0.1	+6.4	+0.1
0.361	103.7±0.2	−0.5	+8.3	+0.3
	121.5±0.2	−0.4	+8.4	+0.2
	147.0±0.2	−0.6	+7.7	+0.2
	167.4±0.2	−0.2	+7.8	+0.0
	192.8±0.2	−0.2	+7.6	−0.1
0.683	103.7±0.2	+0.1	+6.5	+0.6
	121.5±0.2	−0.2	+6.3	+0.2
	147.0±0.2	−0.5	+5.6	−0.3
	167.4±0.2	−0.2	+5.8	−0.0
	192.8±0.2	−0.1	+5.7	0
Mean		−0.4	+6.7	±0.3

As seen from each table, the Kendall formula holds good for AgCl–AgBr and $CdCl_2$–$CdBr_2$ systems, but fails for $PbCl_2$–$PbBr_2$ and Na–K systems, whereas the Ishikawa formula holds good for all the pairs, although the constancy of K in $PbCl_2$–$PbBr_2$ system is somewhat doubtful, yet its superiority to other formulas may be accepted without hesitation.

For $PbCl_2$–$PbBr_2$ system, there exist two different opinions even in these years: Delgery[5] claimed the existence of a stable compound PbClBr and an unstable peritectic compound $PbBr_2 \cdot 3PbCl_2$, but Calingaert, Lamb and Meyer[6] confirmed the former but denied the latter by thermal and x-ray diffraction analyses. The present viscosity analysis gives the result that K in $PbCl_2$(1)–$PbBr_2$(2) system takes a quite different value 3.0 (=1/0.33) as compared with K in AgCl(1)–AgBr(2) and $CdCl_2$(1)–$CdBr_2$(2) systems, taking 0.826 (=1/1.21) and 0.82 respectively.

For Na–K system, a peritectic melting compound Na_2K was confirmed recently by Mckisson and Bromley[7] from the measurement of heat formation. K of this system takes a unique value 2.54 throughout the observed temperature range.

Since K's in mixtures composed of like liquids differ not so much from unity,[8] K's in AgCl–AgBr and $CdCl_2$–$CdBr_2$ systems are regarded as being normal, but those in $PbCl_2$–$PbBr_2$ and Na–K systems as being abnormal. These anomalous values may clearly be explained by the supposition that the tendency of forming a peritectic compound $PbBr_2 \cdot 3PbCl_2$ or Na_2K necessitates the activation of attraction field intensity of lead bromide or potassium to have nearly three times or two times that of its ordinary intensity respectively. These systems, therefore, belong to a quasi-ideal mixture. In fact, in Na–K system occurs slight volume contraction of the simplest form Δv (cc./g.)$=Cz(1-z)$ (z=weight fraction) on mixing, giving C 0.056, 0.051 and 0.048 at 103.7, 147.0 and 192.8 °C. respectively, which, though little in magnitude, suggests a compound of molecular ratio 2Na: K, if it forms.

Summary

Tests made with data in the recent literature show (1) that the Ishikawa formula is superior to the formulas of Macleod, Drucker-Kassel and Kendall which are often used as an ideal mixture law for viscosity, and (2) that the

(5) I. Delgery, *Compt. rend.*, **222**, 886 (1946); **223**, 401 (1946); **224**, 274, 918 (1947).
(6) G. Calingaert, F. W. Lamb and F. Meyer, *J. Am. Chem. Soc.*, **71**, 3709 (1949).
(7) R. L. Mckisson and L. A. Bromley, *J. Am. Chem. Soc.*, **73**, 314 (1951).
(8) T. Ishikawa and T. Baba, This Bulletin, **11**, 64 (1936).

extraordinary values of K of his formula in $PbCl_2$–$PbBr_2$ and Na–K systems suggest the tendency of forming peritectic compounds of $PbBr_2 3PbCl_2$ and Na_2K respectively.

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Surface Chemistry of Synthetic Protein Analogues. I. Surface Pressure-Area Relation of Synthetic Polypeptides as the Model of Proteins

By Toshizo ISEMURA and Kozo HAMAGUCHI

(Received October 8, 1951)

Introduction

Many investigations have been made of the monolayers of proteins at air-liquid or liquid-liquid interfaces to obtain some information about the nature of protein fiims.[1] The surface chemistry of proteins involves many problems to be solved, because of the fact that the proteins are of a rather complicated structure and consisted of various amino-acid residues which are not arranged in orderly manner with uniform distribution along the polypeptide chain. Recently, it became possible to synthesize simple protein analogues of the known structure and of the high molecular weight, by polymerization of N-carboxy-anhydride of α-amino acid.[2] It is well convinced that these synthetic protein analogues play a very important role as a model of natural protein, because of these simple constitutions having one or two known amino acids, though it is unfortunate that the molecular weight distribution is not so uniform as that of natural proteins. It is our aim to obtain some information of natural protein from the surface chemical study of these polymers and to approach to the structure of proteins.

Recently, some interesting studies by Ambrose,[3] Astbury[4] and their collabrators on the synthetic polypeptides by x-ray and infrared analysis have appeared. Whether the results obtained by the monolayer technique would coincide with these by x-ray and infrared data, or not, is also an interesting problem to be investigated.

The synthetic protein analogues examined in the present investigation were poly DL-α-aminocaproic, poly DL-α-aminocaprylic, poly DL-α-aminocapric, poly DL-α-aminolauric acid, and poly γ-methyl-L-glutamate. Poly ε-aminocaproic acid(amilan) was also examined in comparison with the polymers of α-amino acid.

At the first step, surface pressure-area (F-A) relations of the monolayers of these protein analogues on water were examined, and some results obtained were reported in the previous notes.[5],[6] It was found that the F-A curve of poly DL-α-aminocaproic acid which has shorter side chain and those given by natural proteins such as ovalbumin or pepsin resembled very closely in shape. The region which has a very high compressibility appeared in the F-A curves of the polypeptides of which side chains have more than six carbon atoms. It is believed that poly DL-α-aminocaproic, poly DL-α-aminocaprylic, poly DL-α-aminocapric, and poly DL-α-aminolauric acid have a structure of β-keratin type on the surface of water since the area which was found for every polymer to be in the closest packing of polypeptide backbone, was all 14.7 Å.^{2}/amino acid residue. This value is in good agreement with the area calculated by x-ray data for an

(1) J. S. Mitchell, *Trans. Faraday Soc.*, **33**, 1129 (1937); I. Langmuir and V. J. Schaefer, *Chem. Rev.*, **24**, 181 (1939).

(2) R. B. Woodward and C. H. Schramm, *J. Am. Chem. Soc.*, **69**, 1551 (1947); H. Tani, J. Noguchi, *et al.*, *Chem. High Polymers, Japan*, **8**, 51, 53, 57, 62, 65 (1951).

(3) E. J. Ambrose and W. E. Hanby, *Nature*, **163**, 483 (1949); C. H. Bamford, W. E. Hanby and F. Happey, *ibid.*, **164**, 138 (1949); C. H. Bamford, W. E. Hanby and F. Happey, *ibid.*, **164**, 752 (1949); A. Elliot and E. J. Ambrose, *ibid.*, **165**, 921 (1950); C. H. Bamford, W. E. Hanby and F. Happey, *ibid.*, **166**, 829 (1950).

(4) W. T. Astbury, C. E. Dagliesh, S. E. Darmon and G. B. B. M. Sutherland, *Nature*, **162**, 896 (1948); W. T. Astbury, *ibid.*, **163**, 722 (1949); W. T. Asthury, *ibid.*, **164**, 439 (1949).

(5) T. Isemura, K. Hamaguchi, H. Tani, J. Noguchi and H. Yuki, *Nature*, **168**, 165 (1951).

(6) T. Isemura and K. Hamaguchi, *Kagaku* (*Science*), **22**, 87 (1952).

arrangement of β-configuration. Polyγ-methyl-L-glutamate occupied less area per residue than any other polypeptides. It may have a structure of α-keratin configuration. This supposition was comfirmed by the transformation of the film into a film constituted of the molecules of β-configuration by adding some acids to the substrate water.

Experimental

Experimental Method and Materials.—Surface pressure was registered by Sasaki's modification[7] of Harkins' method using the Wilhelmy balance.

The synthetic polypeptides used were prepared by Dr. H. Tani, Dr. J. Noguchi and their collabrators and the samples of amilan were presented by Toyo Rayon Co. The polypeptides were used without any further purification. Amilan was purified by the precipitation from the solution in formic acid by adding water.

Poly DL-α-aminocaproic, poly DL-α-aminocaprylic, poly DL-α-aminocapric, and poly DL-α-aminolauric acids were spread from benzene solution, poly γ-methyl-L-glutamate from pyrididine solution and amilan from a solution in a mixed solvent of cresol and benzene. The molecular weight of the poly DL α-aminolauric acid determined by the osmotic pressure measurement was 172,000. The molecular weight of poly α-aminocaproic acid by the surface technique was 1,060,000.

Results.—The F-A curves obtained with the five polypeptides described above on distilled water are shown in Figs. 1 and 2. The abscissa is the area (m.^{2}) per milligram of polypeptide in Fig. 1 and the area (Å.^{2}) per amino acid residue in Fig. 2. These curves were obtained for the films allowed to stand on water prior to the experiment for six minutes after spreading.

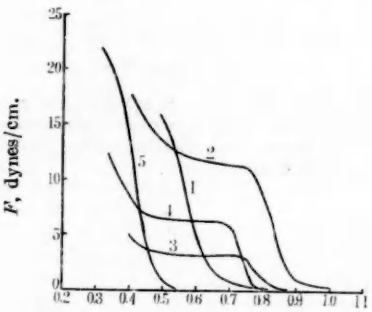

Fig. 1.—The surface pressure-area curves of polypeptide monolayers on distilled water (9°C.): Curve 1, poly DL-α-aminocaproic acid; curve 2, poly DL-α-aminocaprylic acid; curve 3, poly DL-α-aminocapric acid; curve 4, poly DL-α-aminolauric acid; curve 5, poly γ-methyl-L-glutamate.

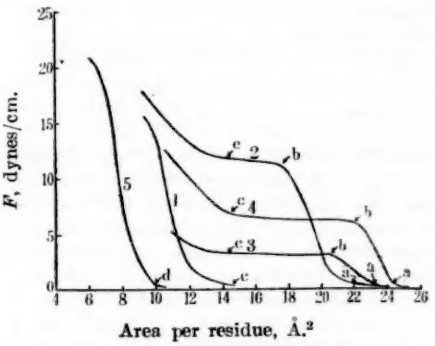

Fig. 2.—The surface pressure-area curves of polypeptide monolayers on distilled water (9°C.).

Poly DL-α-aminocaproic acid (curve 1) occupied the area of 0.68 m.^{2}/mg. (13 Å.^{2}/residue) at 1 dyne/cm., which is in good agreement with the area of 0.7 m.^{2}/mg. (about 14 Å.^{2}/residue) at 1 dyne for the egg albumin or pepsin.[8] The shape of the curve of this polymer was also very similar to that of these natural proteins.

The plateau which is a region of very high compressibility (from point b to c in Fig. 2), appeared in the F-A curves of poly DL-α-aminocaprylic (curve 2), poly DL-α-aminocapric (curve 3), and poly DL-α-aminolauric acid (curve 4). As far as our surface balance of hanging plate type was concerned, the plateau was not recognized for the curve of poly DL-α-aminocaproic acid, but was found to exist in very low pressure region (at about 0.05 dyne/cm.) by using the surface balance of Langmuir-Adam type which has the sensitivity of about 0.001 dyne/cm.

The areas at the points a, b, c, and d which are indicated by arrows for each curve in Fig. 2 are summarized in Table 1. The longer the side chain was, the larger the limiting area (at point a) became. The area at point c was almost the same irrespective of the monomer of α-amino acid. (about 14.7 Å.^{2}/residue). Poly γ-methyl-L-glutamate (curve 5) had a very small limiting area of 9.8 Å.^{2}/residue (at point d).

(7) T. Sasaki, *J. Chem. Soc. Japan*, **62**, 796 (1941).

(8) I. Langmuir and D. F. Waugh, *J. Am. Chem. Soc.*, **62**, 2771 (1940).

Table 1

Polypeptides	Side chain	Area, Å.²/residue Point a	b	c	d
α-Aminocaproic acid	$-(CH_2)_3CH_3$			14.6	
α-Aminocaprylic acid	$-(CH_2)_5CH_3$	22.0	18.0	14.7	
α-Aminocapric acid	$-(CH_2)_7CH_3$	23.0	21.0	14.8	
α-Aminolauric acid	$-(CH_2)_9CH_3$	24.2	22.2	14.7	
γ-methyl-L-glutamate	$-(CH_2)_2COOCH_3$				9.8

The *F-A* curves of poly DL-α-aminocaproic acid and poly γ-methyl-L-glutamate changed little with time after spreading, but the *F-A* curves of the other polypeptides which have longer side chains changed extremely with time. For instance, the effect of aging for poly DL-α-aminocaprylic and poly DL-α-aminolauric acid is shown in Figs. 3 and 4, respectively. The initial spreading concentrations of the former and the latter polymer were 0.51 and 0.48 mg./m.², respectively.

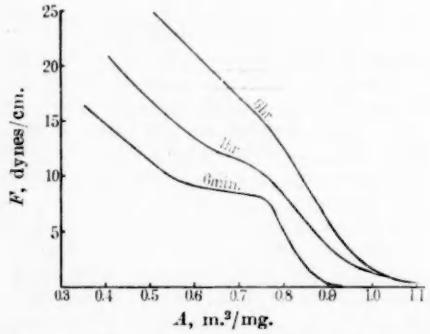

Fig. 3.—Poly DL-α-aminocaprylic acid aged on distilled water (16.5°C.).

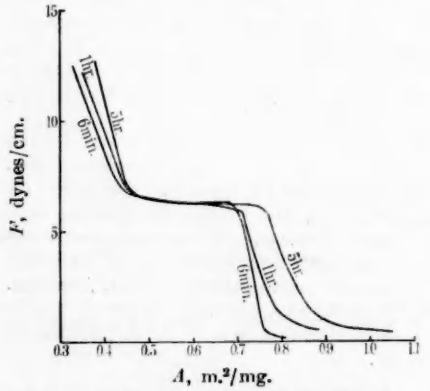

Fig. 4.—Poly DL-α-aminolauric acid aged on distilled water (9°C.).

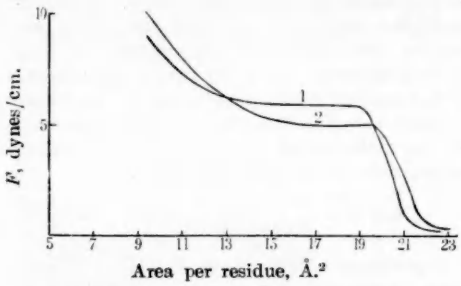

Fig. 5.—Effect of substrate on the acidity of monolayer of poly DL-α-aminocapric acid (21°C.): curve 1, on distilled water; curve 2, on 0.2 *M* formic acid.

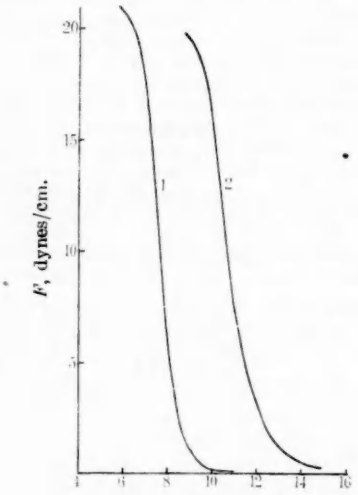

Fig. 6.—Effect of acidity of substrate on the monolayer of poly γ-methyl-L-glutamate (22°C.): curve 1, on distilled water; curve 2, on 0.02 *M* formic acid.

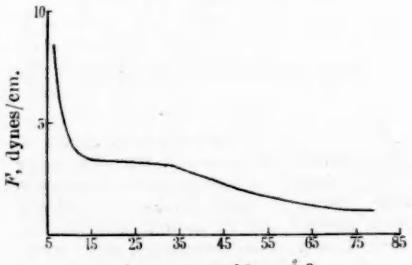

Fig. 7.—The surface pressure-area curve of poly ε-aminocaproic acid. (23°C.)

Acidic substrate exerted no detectable effect upon the monolayers of poly DL-α-aminocaprylic, poly DL-α-aminocapric and poly DL-α-aminolauric acid. For instance, the effect of 0.2 *M* formic acid on the monolayer of poly DL-α-aminocapric acid is shown in Fig. 5. The *F-A* curve of the monolayer on acidic substrate is shown by curve 2, together with the curve on distilled water (curve 1) in the figure.

On the other hand, when poly γ-methyl-L-glutamate was spread on acidic substrate, the limiting area was increased to larger area than on distilled water. Curve 2 in Fig. 6 shows *F-A* curve of the polymer on acidic substrate containing formic acid in concentration of 0.02 *M*. For comparison with the monolayer on acidic substrate, the curve of the monolayer on distilled water is shown by curve 1 in the figure. Using acetic or hydrochloric acid solution as substrate, the curves were practically the same as curve 2. The limiting area of the curve on these acid substrate was also 14.7 Å.^{2}/residue, corresponding to the area of the other polypeptides at point c in Table 1.

In Fig. 7, the *F-A* curve of amilan on distilled water is shown. The polymer of ε-amino acid gave the curve of an expanded type which was entirely different from those of the polymers of α-amino acid.

Discussion

As pointed out above, the *F-A* curve of poly DL-α-aminocaproic acid resembled those of natural proteins such as the egg albumin or pepsin, the reason of which may be that the carbon number of hydrocarbon side chain of the polymer (four) is very near to the average carbon number of side chain of natural proteins, that is, about three. The preliminary experiments on the surface viscosity of this polymer also bore a close resemblance to those of natural proteins.[9]

The plateau which is a region of very high compressibility was also found in the *F-A* curve of poly DL-α-aminocaproic acid at very low pressure. The results obtained by H. B. Bull from the experiments on the egg albumin[10] showed the existence of the plateau in very low pressure region. These facts also show the resemblance between the synthetic polypeptides having shorter side chains and natural proteins.

The curves for the other polypeptides having longer side chains had a plateau in higher pressure region, which is not found in the case of natural proteins. This fact may be attributed to the absence of longer side chains with more than six carbon atoms on the average.

At areas larger than that indicated by point a (Fig. 2), the polypeptide molecules are spread with chains flat on the surface. Between points a and b on the *F-A* curves, the surface pressure would be exerted mainly by the action of side chains. Surface viscosity measurement showed that the viscosity in this region was as large as that of the state of gel. From point b to c, the surface pressure was kept practically constant. It suggests that the molecules of polypeptide would be reorientated in this region by compression and the side chains would be compressed away from the surface, so that they might be forced upwards or downwards into the water. Thus, at point c, a film which is arranged in close packed polypeptide backbone of β-configuration would be formed, since the area at this point for every polymer was found to be 14.7 Å.^{2}/amino acid residue, and this value corresponds to the area calculated with the x-ray data for an arrangement of β-configuration. When the surface viscosity against area curve was registered for the monolayer of poly DL-α-aminocaproic acid, the sudden increase in slope of the curve occurred at 14.7 Å.^{2}/residue. The same value was found by Cumper and Alexander[11] from their investigation on the surface viscosity for polyalanine (at air-liquid and liquid-liquid interfaces) and polyphenylalanine (at air-liquid interface). From point c, the pressure was again increased by further compression. It might be considered that segments of the chain might be left the surface.

Polyorganosiloxanes,[12] Buna N,[13] and long chain urea derivatives[14] give the similar *F-A* curves as those of polypeptides with longer side chains, which have the plateau in the curve. The effect of temperature on these monolayers is also similar to that on the polypeptides. The higher the temperature of substrate is, the situation of the plateau passes to the lower pressure region. Fox, Taylor and Zisman[12] characterized the appearance of this plateau as the process of coiling of high linear

(9) M. Joly, *Research*, Supplement, "Surface Chemistry," Butterworth, 1949, p. 157.

(10) H. B. Bull, *J. Biol. Chem.*, **185**, 27 (1950).

(11) C. W. N. Cumper and A. E. Alexander, *Trans. Faraday Soc.*, **46**, 235 (1950).

(12) H. A. Fox, P. W. Taylor and W. A. Zisman, *Ind. Eng. Chem.*, **39**, 1401 (1947).

(13) K. Suzuki, *Koshitugaku-Ronso (Discussions Colloid Sci., Japan)*, **1**, 63 (1947).

(14) J. Glazer and A. E. Alexander, *Trans. Faraday Soc.*, **47**, 401 (1951).

polymer molecule by compression on the surface of water. Saraswat and Kalyanasundarm[15] presented the same idea. As far as the synthetic polypeptides are concerned, however, the process of coiling of the linear molecule would not be considered as the reason of the appearance of the plateau, because, contrary to polyorganosiloxanes, the longer the side chains of the polypeptides are, the more marked the existence of the plateau became and it would be impossible for polypeptide molecules of which side chains have six, eight, or ten carbon atoms to be coiled by compression. Merely, from the resemblance of the shape of *F-A* curves, it can hardly be concluded that the properties of the monolayers of polyorganosiloxanes and of polypeptides would be the same. From both points of view of chemical structure in which —CO—NH— group is contained and of the behaviors of monolayers, the most closely related films to that of polypeptide may be the films of hexadecyl and octadecyl urea which wer einvestigated by Glazer and Alexander[14]. The shape of *F-A* curves of these urea derivatives resembles closely those of polypeptides, and also it is the same for both substances that the physical state from point a to b is gel or solid. However, that the physical state of higher pressure region above point c is liquid in the case of urea derivatives, is different from those of polypeptides. They considered in the case of urea derivatives that the molecules are combined by hydrogen bonding with each other in the region from point a to b, and that in higher pressure region above point c, no interchain hydrogen bonding exists. Therefore, the appearance of the plateau was considered to be due to the breaking of hydrogen bonds. In the monolayer of synthetic polypeptides, however, there occurred reorientation of the molecules in the region from point b to c, being accompanied by the formation of inter-chain hydrogen bonds. The molecules are bound strongly by inter-chain hydrogen bond if the monolayer was sufficiently compressed, so that polypeptide could be drawn out as a string from the surface of water by a glass rod. This is in good agreement with the experimental results that the area at point c at which interchain hydrogen bonding may be completed are always 14.7 Å.^{2}/amino acid residue, regardless of the lengths of the side chains.

As pointed out above, the monolayer of poly γ-methyl-L-glutamate gave much smaller limiting area than the other polypeptides. It gave the limiting area of 9.8 Å.^{2}/residue on distilled water but this limiting area was increased to 14.7 Å.^{2} on acidic substrate. The difference of the limiting area dependent on the nature of the substrate may be due to the change of the configuration of the molecule. Some considerations were presented by us previously.[6]

It is known from the results of infra-red analyis by Ambrose *et al.* that poly γ-methyl-L-glutamate has a structure of α-configuration and is not transformed to β-configuration even if it is forcibly stretched.[16] Protein molecules having a folded structure would be generally unfolded on the surface of water probably transforming to β-type, since interface such as the surface of water exerts a strong force on the molecules. If the model of α-configuration for proteins, presented by Shimanouchi and Mizushima[17] and by Ambrose *et al.*[16] independently, is accepted, the area per amino acid residue when molecules are arranged in α-configuration must be two thirds of the area occupied by the molecule arranged in β-configuration. On the other hand, if the model of α-configuration presented by Astburty,[18] is accepted, the area occupied by the molecule of α-type must be half the area in the case of β-configuration. The experimental results obtained with this polymer are in good agreement with the former case (14.7 Å.^{2}×2/3=9.8 Å.^{2}). It is known that poly γ-methyl-L-glutamate having α-configuration is transformed to β-configuration by the treatment with formic or acetic acid.[19] When poly γ-methyl-L-glutamate was spread on the substrate containing hydrochloric, formic, or acetic acid, the surface pressure against the area curve shown by curve 2 in Fig. 6 was obtained, and the limiting area became to 14.7 Å.^{2}/residue, *i. e.*, the limiting area of the polypeptides having β-configuration. These results correspond to the α→β transformation due to the destruction of the intra-chain hydrogen bonding by the interaction of the polypeptide with the acids in substrate. The results obtained with the surface viscosity measurement, the details of which will be reported elsewhere, led to the same conclusion. On distilled water the surface viscosity of the monolayer formed by polypeptide of α-configuration is considerably greater than that of proteins even at lower pressure region and suddenly increased at the area of about 9.8

(15) H. C. Saraswat and A. Kalyanasundarm, *J. Polymer Sci.*, **6**, 82 (1951).

(16) E. J. Ambrose and W. E. Hanby, *Nature*, **163**, 483 (1949).

(17) T. Shimanouchi, and S. Mizushima, *Science (Japan)*, **17**, 24, 52 (1947); This Bulletin, **21**, 1 (1948).

(18) W. T. Astbury and F. O. Bell, *Nature*, **147**, 696 (1941).

(19) C. H. Bamford, W. E. Hanby and F. Happey, *Nature*, **166**, 829 (1950).

Å.²/residue. On acidic substrate, however, the surface viscosity increased steeply at about 14.7 Å.²/residue and at the area larger than this, the polymer exhibited the viscosity of the same order of magnitude as that of any other polypeptides. Moreover, acidic substrates exerted little effect on the monolayer of poly DL-α-aminocapric acid, which spread as a film of β-configuration as shown in Fig. 5. These facts must be the surface-chemical indications of the $\alpha \rightarrow \beta$ transformation of poly γ-methyl-L-glutamate.

The reason for the change of *F-A* curve of the monolayers of polypeptides having longer side chains as a function of time after spreading is not yet clear. This phenomena, however, may probably be caused by the decrease of the entanglement of the side chains of polypeptide molecules. The effect of aging on the monolayer of polyorganosiloxanes observed by Fox, Solomon and Zisman[20] are apparently analogous to those of the polypeptides. They considered that this effect may be due to the hydrolysis of polyorganosiloxanes by the interaction with substrate. The reason for the aging considered by them, however, may not be valid for the present case.

As previously mentioned, the behaviors of monolayers of the synthetic polypeptides are much influenced by inter and intra-chain hydrogen bonding. From this reason, the monolayer of poly ε-aminocaproic acid, was also examined, in which five methylene groups are placed between NH- and CO-groups. The *F-A* curve of this polymer is of very expanded type and is entirely different from that of the polymers of α-amino acid. It is concluded from this point of view that the poly ε-aminocaproic acid is inadequate as a model of natural proteins. Detailed consideration about this polymer will be reported elsewhere.

Summary

The surface pressure against area (*F-A*) curves of the monolayers of poly-DL-α-aminocaproic, poly DL-α-aminocaprylic, poly DL-α-aminocapric, poly DL-α-aminolauric acids, poly γ-methyl-L-glutamate and poly ε-aminocaproic acid (amilan) was measured. The results obtained were as follows:

(1) The *F-A* curve of poly DL-α-aminocaproic acid having four carbon atoms in the side chain resembled in shape very closely to those given by natural proteins such as the egg albumin or pepsin. This fact is due to the resemblance of the carbon number in the side chains of both of the polypeptide and proteins.

(2) The plateau which is a region of very high compressibility appeared in the *F-A* curves of the polypeptides having more than six carbon atoms in the side chains. The curves of these polypeptides were entirely different from those of natural proteins.

(3) The area per amino acid which was found for every polypeptide to be in the closest packing of polypeptide backbone was all 14.7 Å.² This value is in good agreement with the area calculated from x-ray data for an arrangement of β-keratin configuration.

(4) Poly γ-methyl-L-glutamate occupied the limiting area of 9.8 Å.²/amino acid residue on distilled water. This value can be sufficiently accounted for, if this polymer has a structure of α-configuration on water surface and the model of α-keratin configuration presented by Shimanouchi and Mizushima and by Ambrose *et al.* is accepted. On substrates containing hydrochloric, formic, or acetic acid, however, this polypeptide occupied the limiting area of 14.7 Å.²/residue, this value corresponding to the area of the other polypeptides which are arranged in close packed β-configuration. The $\alpha \rightarrow \beta$ transformation of this polypeptide could be demonstrated by monolayer technique.

(5) The monolayer of the polymer of ε-aminocaproic acid is inadequate as a model of protein films.

The authors wish to express their sincere thanks to Dr. H. Tani in Faculty of Science, Osaka University and to Dr. J. Noguchi at Kanazawa University who gave them the valuable samples of synthetic polypeptides. A part of the expense for the experiment has been defrayed from a grant given by the Ministry of Education, to which the authors' thanks are due.

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The Relation between the Geometrical Configurations of Inorganic Complexes and their Absorption Bands. II.[1] The Configurations of Two Isomers of Sodium Disulfono-tetrammine-cobaltiate

By Yoichi SHIMURA

(Received October 9, 1951)

The two isomers of $Na[Co(NH_3)_4(SO_3)_2]$ were first prepared by Hofmann and Jenny,[2] who designated a brown, more insoluble dihydrate as *cis*-form and another golden yellow, more soluble tetrahydrate as *trans*-form. But this assignment was unreliable, since it was deduced from an uncertain analogy with two isomers of dinitro-tetrammine-cobalt(III) salt.

As will be shown in this report, however, the spectral data prove that the brown salt is *trans*-isomer and the golden yellow salt *cis*-isomer. There is, of course, a possibility of other isomerism, *i. e.*, sulfono-sulfito isomerism, which corresponds to nitro-nitrito isomerism of the complexes containing some NO_2^--radicals. In such case, the difference between both isomers consists in the difference of the atom which combines directly with the central cobalt (III) ion: in sulfono-isomer the S-atom of $SO_3^=$-radical is bound to the cobalt and in sulfito-isomer the O-atom is bound to it. But the spectral data exclude this possibility as will be seen later.

Results and Discussion

The absorption spectra of the two salts were measured at the spectral region from 55 to 125×10^{13}/sec. in 3 % aqueous solutions of NH_3. The absorption curves are shown in Fig. 1.

The brown salt has three absorption maxima in this region and the yellow salt only two maxima. It has been well known[3] that the complex ions with a pair or pairs of negative ligands in *trans*-positions have the third absorption bands, which are situated almost always in the region $110\text{–}120\times10^{13}$/sec. for Co (III) or Cr (III) complex salts. Accordingly, the brown salt which has a third band at 113.6×10^{13}/sec. is *trans*-isomer concerning the positions of $SO_3^=$-radicals, and the yellow salt which has no absorption band in the region of $110\text{–}120\times10^{13}$/sec. is *cis*-isomer.

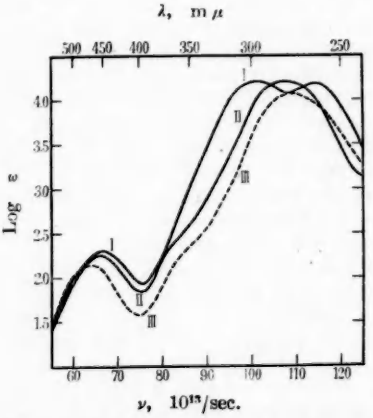

Fig. 1.—Absorption spectra of: I, brown salt, $Na[Co(NH_3)_4(SO_3)_2]\cdot2H_2O$ (*trans*) in 3 % NH_3 aq.; II, golden yellow salt, $Na[Co(NH_3)_4(SO_3)_2]\cdot4H_2O$ (*cis*) in 3 % NH_3 aq.; III, $[Co(NH_3)_5SO_3]Cl$ (Kiss and Czeglédy).

The larger absorption bands, which are distributed at 101.0×10^{13}/sec. for *trans*-compound and at 107.8×10^{13}/sec. for *cis*-compound, are the "$SO_3^=$-specific absorption bands" due to the coördinated $SO_3^=$-radicals. The absorption spectra of $[Co(NH_3)_5SO_3]Cl$ (Fig. 1, Curve III) and $Na_5[Co(CN)_4(SO_3)_2]$, which have been reported by Kiss and his co-workers,[4],[5] have also the similar $SO_3^=$-specific bands.

The $SO_3^=$-specific band of the *trans*-salt is more bathochromic than that of the *cis*-salt. In a previous paper,[1] the author has pointed out that the rhodanato- or nitro-specific bands

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(2) K. A. Hofmann and A. Jenny, *Ber.*, **34**, 3855 (1901).
(3) R. Tsuchida, This Bulletin, **13**, 388, 436 (1938).
(4) Á. v. Kiss and D. v. Czeglédy, *Z. anorg. Chem.*, **235**, 407 (1938).
(5) Á. v. Kiss, G. Auer and E. Major, *ibid.*, **246**, 28 (1941).

Table 1

First Band of $[Co(NH_3)_4A_2]$

Spectrochemical series of ligands	Complex ion	Position of first band, 10^{13}/sec.	Ref.
NO_2^-	*trans*-$[Co(NH_3)_4(NO_2)_2]^+$	68.2,[a] 68.9	(6), (7)
	cis-$[Co(NH_3)_4(NO_2)_2]^+$	67.0,[a] 69.0	(6), (7)
$SO_3^=$	*trans*-$[Co(NH_3)_4(SO_3)_2]^-$	66.6 [b]	
	cis-$[Co(NH_3)_4(SO_3)_2]^-$	65.9 [b]	
NH_3	$[Co(NH_3)_4(NH_3)_2]^{3+}$	63.4	(7)
H_2O	$[Co(NH_3)_4(H_2O)_2]^{3+}$	60.0	(8)
$C_2O_4^=$	$[Co(NH_3)_4C_2O_4]^+$	58.8	(7)
$CO_3^=$	$[Co(NH_3)_4CO_3]^+$	58.4	(7)
Cl^-	*trans*-$[Co(NH_3)_4Cl_2]^+$	<50	(8)

(a) in methanol-water solution. (b) in 3% NH_3 aq.

of *trans*-forms of Co(III) complexes have the longer wave-lengths than those of the *cis*-forms. Now it is recognized that such regularity is also true for the $SO_3^=$-specific absorption bands.

It has been shown by Tsuchida[3] that the near infra-red, the visible and the ultra-violet absorption bands of the complex salts are classified by their origins into the first, second, third and specific absorption bands. As is clear from Fig. 1, each of the isomers has the first band, but the second bands are inexplicit, since those are hidden under the larger $SO_3^=$-specific bands. The first band of the *trans*-salt is slightly shifted to the shorter wave-length than that of the *cis*-salt. In this connection, the relationship of the first band to the *cis-trans* isomerism will be discussed in the third report of this series. A small inflexion of the absorption curve of the *cis*-form at about 82×10^{13}/sec. is attributed to the second band. A similar hump is found in the absorption curve of the pentammine salt.

To determine the position of $SO_3^=$-radical on the spectrochemical series,[3] $[Co(NH_3)_4A_2]$ type complex ions (A=a molecule or a negative ion) were arranged in Table 1 in the order of frequencies of their first bands. From the table, $SO_3^=$-radical is located between NO_2^- and NH_3 on the series. This fact indicates that $SO_3^=$-radical coördinates around the central Co(III) ion by the lone pair of electrons of its S-atom, since it is expected that if $SO_3^=$-radical is bound to cobalt by O-atom, its position on the series should be behind NH_3. For instance, NO_2^--radical is ahead of NH_3 on the series when it coördinates by its lone pair electrons of N-atom, while it is behind NH_3 when it coördinates by O-atom forming the nitrito-complexes. The *cis*- and *trans*-salts studied in this paper, therefore, should be named sodium disulfono-tetrammine-cobaltiate, instead of disulfito-tetrammine-cobaltiate.

Moreover, the above discussion is confirmed from the following consideration. The absorption spectrum of a free $SO_3^=$-ion has a maximum at 100×10^{13}/sec.[4] All of the $SO_3^=$-specific bands of the complexes given in Fig. 1, are distributed in the more hypsochromic region than the absorption maximum of the free $SO_3^=$-ion. This shows that the coördinated $SO_3^=$-radical is more stable than a free $SO_3^=$-ion. $SO_3^=$-ion has a pyramidal structure, in which 3 $O^=$-ions and a lone pair of electrons are bound tetrahedrally around the central sulfur ion as $\left[\begin{matrix}O\\|\\:S-O\\|\\O\end{matrix}\right]^=$, whilst this lone pair of S-atom is utilized in sulfono-Co (III) complexes, by which the structure should be compared to a sulfate type, *i. e.*, $\left[\begin{matrix}O\\|\\Co-S-O\\|\\O\end{matrix}\right]$. In this manner, the sulfono-radical in coördination is more symmetrical and more stable than a free $SO_3^=$-radical. Thus the hypsochromic shift of the absorption band of $SO_3^=$ is observed. If the radical is coördinated by O-atom as $\begin{matrix}Co-O\\\diagdown\\\quad S\quad:\\\diagup\quad\diagdown\\O\qquad O\end{matrix}$, this sulfito-radical in coördination becomes more unstable than a single $SO_3^=$-ion and in consequence the "sulfito-specific band" should appear in the long wave-length side as compared with the absorption maximum of a free $SO_3^=$-ion. Therefore, the specific bands given in Table

(6) F. Basolo, *J. Am. Chem. Soc.*, **72**, 4393 (1950).
(7) H. Kuroya, *J. Inst. Polytech., Osaka City Univ.*, **1**, No. 1, Ser. C, 29 (1950).
(8) R. Tsuchida, This Bulletin, **11**, 721 (1936).

2a, are undoubtedly the "sulfono-specific bands", and these salts are the disulfono-tetrammine- or sulfono-pentammine-cobaltiate. This is also clear from comparing the Table 2a with Table 2b.

Table 2

a: Position of Sulfono- or Sulfito-specific Absorption Band

	$\times 10^{13}$/sec.	Ref.
Sulfito-specific band	(smaller than 100)	
Absorption maximum of a free $SO_3^{=}$-ion	100($\log \varepsilon = 1.0$)	(4)
Sulfon-specific band:		
trans-$Na[Co(NH_3)_4(SO_3)_2]$	101.0($\log \varepsilon = 4.22$)	
cis-$Na[Co(NH_3)_4(SO_3)_2]$	107.8($\log \varepsilon = 4.21$)	
$[Co(NH_3)_5SO_3]Cl$	109.5($\log \varepsilon = 4.08$)	(4)

b: Position of Nitro- or Nitrito-specific Absorption Band

	$\times 10^{13}$/sec.	Ref.
Nitrito-specific band	83–84	(7)
Absorption maximum of a free NO_2^--ion	84.5($\log \varepsilon = 1.3$)	(9)
Nitro-specific band:		
trans-$[Co(NH_3)_4(NO_2)_2]Cl$	86.8($\log \varepsilon = 3.90$)	(7)
cis-$[Co(NH_3)_4(NO_2)_2]NO_3$	91.5($\log \varepsilon = 3.90$)	(7)
$[Co(NH_3)_5NO_2]SO_4$	91.3($\log \varepsilon = 3.57$)	(7)

Structure of $NH_4[Co(NH_3)_4(SO_3)_2]\cdot 3H_2O$—Two isomers of $NH_4[Co(NH_3)_4(SO_3)_2]$ have also been known, *i. e.*, a dark brown trihydrate and other reddish brown tetrahydrate. According to Hofmann and Jenny,[2] the dark brown isomer corresponds to the brown sodium salt and the reddish brown isomer to the golden yellow sodium salt. From the data of the photochemical decompositions of the salts, Schwarz and Tede[10] supported the theory that the dark brown salt is *cis*-isomer and the reddish brown salt is *trans*-isomer. Klement,[11] and Bailar and Peppard[12] also concluded, from the studies of reactions starting from the dark brown trihydrate, that the salt has two $SO_3^{=}$-groups in *cis*-positions. But the conclusions are not very reliable, since some rearrangements of ligands might have taken place in the course of such reactions.

The absorption spectrum of the dark brown isomer, however, is almost identical to that of the brown sodium salt (Table 3). This suggests that the salt is ammonium *trans*-disulfono-tetrammine-cobaltiate, although it is contrary to the several investigators' assignments of *cis*-configuration, and to solve this contradiction, it is hoped that the detailed reaction mechanism of the salt will be studied.

Experimental

Materials—*trans*-$Na[Co(NH_3)_4(SO_3)_2]\cdot 2H_2O$(brown salt) and *cis*-$Na[Co(NH_3)_4(SO_3)_2]\cdot 4H_2O$ (golden yellow salt) were partly prepared by the method of Hofmann and Jenny,[2] and partly by a modification of that as follows: 21 grams of anhydrous cobaltous carbonate were dissolved in 250 ml. of *ca.* 30% acetic acid and mixed with 250 ml. of 28% NH_3 aq. A vigorous stream of air was drawn through the solution for 24 hours. Through the resultant solution gaseous sulfur dioxide was passed until a considerable amount of brownish orange precipitates appeared. After removal of the precipitates, a mixture of 38 grams sodium bisulfite dissolved in 100 ml. of water and 100 ml. of 28% NH_3 aq. was added to the filtrate. Leaving the mixture for 4 hours at room temperature, the precipitated dark yellow powder was removed by filtration. The filtrate, on cooling in water for 6 hours, precipitated the brown crystals of *trans*-$Na[Co(NH_3)_4(SO_3)_2]\cdot 2H_2O$. Then it precipitated, by cooling in ice for 10 hours, first a little amount of *cis-trans* mixtures and then the almost pure golden yellow crystals of *cis*-$Na[Co(NH_3)_4(SO_3)_2]\cdot 4H_2O$. The rough products of both the isomers were recrystallized from concentrated aqueous solutions by addition of 95% methanol. These were washed by methanol and dried in air. The absorption spectra of the two salts which were obtained by the above method com-

Table 3

Absorption Maxima of Three Disulfono-complexes

Complex salt	First band		Second band		Third band		Sulfono-specific band	
	ν_1, 10^{13}/sec.	$\log \varepsilon_1$	ν_2, 10^{13}/sec.	$\log \varepsilon_2$	ν_3, 10^{13}/sec.	$\log \varepsilon_3$	ν_s, 10^{13}/sec.	$\log \varepsilon_s$
trans-$Na[Co(NH_3)_4(SO_3)_2]\cdot 2H_2O$ (brown salt)	66.6	2.32	?		113.6	4.19	101.0	4.22
cis-$Na[Co(NH_3)_4(SO_3)_2]\cdot 4H_2O$ (golden yellow salt)	65.9	2.26	?		——	——	107.8	4.21
$NH_4[Co(NH_3)_4(SO_3)_2]\cdot 3H_2O$ (dark brown form)	66.6	2.35	?		114.2	4.20	101.2	4.22

(9) H. Kuroya and R. Tsuchida, *J. Chem. Soc. Japan*, **59**, 1142 (1938).
(10) R. Schwarz and K. Tede, *Ber.*, **60**, 63 (1927).
(11) R. Klement, *Z. anorg. Chem.*, **150**, 117 (1926).
(12) J. C. Bailar, Jr. and D. F. Peppard, *J. Am. Chem. Soc.*, **62**, 105 (1940).

pletely accord with those of the salts obtained by the original direction of Hofmann and Jenny.

The dark brown form of ammonium disulfonotetrammine-cobaltiate, $NH_4[Co(NH_3)_4(SO_3)_2]\cdot 3H_2O$, was prepared by the method of Bailar and Peppard.[12]

Spectrographic Measurements—The formal extinction coefficient ε was calculated from the equation: $I=I_0\times 10^{-\varepsilon cd}$, where c is the concentration of the complex ion in gram-ion per liter and d the thickness of the layer in cm. The measurements were made in 3% aqueous solutions of NH_3, because aqueous solutions of the disulfono-complexes because turbid when allowed to stand for about 30 minutes in an open place, while in 3% NH_3 aq. the clearness could be kept almost perfectly for a long time. The comparison cell was also filled with 3% NH_3 aq. Measurements were done at room temperature, with concentrations of 2.5×10^{-3} and 1.0×10^{-4} gram-ion per liter and with varying thickness from 5 to 1 cm.

Summary

The visible and ultra-violet absorption spectra of $Na[Co(NH_3)_4(SO_3)_2]\cdot 2H_2O$ (brown salt) and $Na[Co(NH_3)_4(SO_3)_2]\cdot 4H_2O$ (yellow salt) have been measured. The results have proved that the $SO_3^{=}$-radical coördinates around the central cobalt (III) ion by the lone pair electrons of its sulfur atom, and that the brown salt has *trans*-configuration concerning the positions of the $SO_3^{=}$-radicals and the yellow salt *cis*-configuration.

The absorption spectrum of $NH_4[Co(NH_3)_4(SO_3)_2]\cdot 3H_2O$ (dark brown form) has also been studied and it has been assumed that the salt is *trans*-isomer contrary to several investigators' assignments of *cis*-structure.

The author expresses sincere thanks to Professor R. Tsuchida for his kind advice and encouragement.

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The Relation between the Geometrical Configurations of Inorganic Complexes and their Absorption Bands. III.[1] A General Rule for the First and Second Bands of Co (III) Complexes

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(Received October 12, 1951)

Introduction

Many data have been reported in scientific literature about the absorption spectra of the geometrical isomers of Co(III) complex salts, but the relation between the *cis-trans* isomerism of the complexes and their absorption bands has not been completely clarified. It is well known that the visible and ultra-violet absorption bands of Co(III) complexes consist of the first, second, third and some other specific bands. The relations of these bands to the geometrical configurations, which have already been offered by some authors, are summarized as follows.

(1) **Third band.**—According to Shibata-Tsuchida's *trans*-anions hypothesis,[2] the Co(III) complexes which contain a pair or pairs of negative ligands in *trans*-positions are expected to have a third band at the region of 110-120 $\times 10^{13}$/sec. More recently, Basolo[3] reported the third bands of some *cis*-anions complexes in the shorter wave-lengths than the corresponding *trans*-isomers. These facts are applicable to the configurational determination of the complex salt.

(2) **Specific bands.**—The bands are due to the special ligands in the coördination sphere. In the previous reports,[4],[1] the author has shown that the nitro-, rhodanato- and sulfono-

(1) Part II of this report, Y. Shimura, This Bulletin, **25**, 46 (1952).

(2) R. Tsuchida, *ibid.*, **13**, 388, 436 (1938).
(3) F. Basolo, *J. Am. Chem. Soc.*, **72**, 4393 (1950).
(4) Y. Shimura, *ibid.*, **73**, 5079 (1951).

specific bands of the *trans*-isomers of Co(III) complexes have always the longer wave-lengths than those of the *cis*-forms. This relationship is also useful for the configurational determination of complex salt.

(3) **First band.**—The band is always in the visible region for Co(III) complexes and the color of the complex salt is due to the band. It has frequently been stated that the *cis*-isomers are more highly colored than the *trans*-isomers, but this rule is not reliable since many exceptions are known.

(4) **Second band.**—According to Tsuchida,[2] this band, which appears in the near ultra-violet region for Co(III) complexes, gives a measure of stability of the complex. Basolo[3] stated that the second band of *cis*-form is shifted towards the shorter wave-length as compared with that of *trans*-form, even though his "second band" is confused with the nitro- or rhodanato-specific band.

At the same time, Basolo assumed that whenever the stability differences of stereoisomers are sufficiently large to cause a shift in the absorption band positions, then the one that absorbs at the shorter wave-length is the *cis*-isomer. But it must be noticed here that in fact the first bands of *trans*-compounds containing some nitro- or sulfon-radicals are almost always shifted slightly more towards the shorter wave-lengths than the corresponding *cis*-compounds.

Thus, the relation of the third and some

Table 1

First Band of $[Co\ en_2\ A_2]$ or $[Co(NH_3)_4A_2]$ Type Complex Ion

No.	Spectrochemical series of ligand A	Complex ion	First band (10^{13}/sec.) of: *cis*-isomer, ν_c	*trans*-isomer, ν_t	$\nu_c-\nu_t$	Solvent	Ref.
1	NO_2^-	$[Co(NH_3)_4(NO_2)_2]^+$	67.0	68.2	−1.2	methanol-water	Basolo[3]
1a	NO_2^-	$[Co(NH_3)_4(NO_2)_2]^+$	65.5	67.9	−2.4	water	Kuroya and Tsuchida[5]
1b	NO_2^-	$[Co(NH_3)_4(NO_2)_2]^+$	69.0	68.9	+0.1	water	Kuroya[6]
2	NO_2^-	$[Co\ en_2\ (NO_2)_2]^+$	69.0	69.9	−0.9	water	Kuroya and Tsuchida[5]
2a	NO_2^-	$[Co\ en_2\ (NO_2)_2]^+$	68.5	69.3	−0.8	methanol-water	Basolo[3]
3	$SO_3^=$	$[Co(NH_3)_4(SO_3)_2]^-$	65.9	66.6	−0.7	3%-NH_3 aq.	Shimura[1]
4	NH_3	$[Co\ en_2\ (NH_3)_2]^{3+}$	63.8	63.8	0.0	water	Basolo[3]
5	H_2O	$[Co\ en_2\ (H_2O)_2]^{3+}$	60.6	60.4	+0.2	water *p*H 0.7	Basolo[3]
6	NCS^-	$[Co\ en_2\ (NCS)_2]^+$	59.3	59.0	+0.3	water	Shimura[4]
6a	NCS^-	$[Co\ en_2(NCS)_2]^+$	57.7	58.3	−0.6	methanol-water	Basolo[3]
7	Cl^-	$[Co(NH_3)_4Cl_2]^+$	(57.3)[a]	<50.0	>+7.3	water	Tsuchida[8]
8	Cl^-	$[Co\ en_2\ Cl_2]^+$	56.6	48.0	+8.6	methanol-water	Basolo[3]
8a	Cl^-	$[Co\ en_2\ Cl_2]^+$	55·8	48.4	+7.4	alcohol-water	Ernsberger and Brode[7]
9	Br^-	$[Co\ en_2\ Br_2]^+$	54.5	45.6	+8.9	alcohol-water	Ernsberger and Brode[7]

(a) datum of the crystal.[9]

(5) H. Kuroya and R. Tsuchida, This Bulletin. **15**, 427 (1940).
(6) H. Kuroya, *J. Inst. Polytech., Osaka City Univ.*, **1**, No. 1, Ser. C, 29 (1950).
(7) M. L. Ernsberger and W. R. Brode, *J. Am. Chem. Soc.*, **56**, 1842 (1934).
(8) R. Tsuchida, This Bulletin, **11**, 721 (1936).
(9) R. Tsuchida and M. Kobayashi, *ibid.*, **13**, 619(1938).

Table 2

First Band of [Co en_2 A·B] or [Co$(NH_3)_4$ A·B] Type Complex Ion[a]

No.	Spectrochemical series of ligand B	Complex ion	First band (10^{13}/sec.) of: *cis*-isomer, ν_c	*trans*-isomer, ν_t	$\nu_c - \nu_t$	Solvent	Ref.
	(a) Monochloro-bisethylenediamine or -tetrammine series (A = Cl^-)						
1	NO_2^-	[Co en_2 Cl·NO_2]$^+$	64·5	64.4	+0.1	methanol-water	Basolo[3]
2	NCS$^-$	[Co en_2 Cl·NCS]$^+$	59.6	54.0	+5.6	water	Kuroya and Tsuchida[5]
2a	NCS$^-$	[Co en_2 Cl·NCS]$^+$	58.8	57.7	+1.1	methanol-water	Basolo[3]
3	H_2O	[Co$(NH_3)_4$Cl·H_2O]$^{2+}$	58.5	50.0	+8.5	water	Tsuchida[5]
4	Cl^-	[Co en_2 Cl·Cl]$^+$	56.6	48.0	+8.6	methanol-water	Basolo[3], (b)
5	Br^-	[Co en_2 Cl·Br]$^+$	55.1	47.0	+8.1	alcohol-water	Ernsberger and Brode[7]
	(b) Mononitro-bisethylenediamine or -tetrammine series (A = NO_2^-)						
1	NO_2^-	[Co$(NH_3)_4$$NO_2$·$NO_2$]$^+$	67.0	68.2	−1.2	methanol-water	Basolo[3], (b)
2	NO_2^-	[Co en_2 NO_2·NO_2]$^+$	69.0	69.9	−0.9	water	Kuroya and Tsuchida[5], (a)
3	NCS$^-$	[Co$(NH_3)_4$$NO_2$·NCS]$^+$	62.6	64.0	−1.4	water	Shimura (present paper)
4	NCS$^-$	[Co en_2 NO_2·NCS]$^+$	64.8	65.3	−0.5	water	Kuroya and Tsuchida[5]
5	Cl^-	[Co en_2 NO_2·Cl]$^+$	64.5	64.4	+0.1	methanol-water	Basolo[3]
	(c) Monorhodanato-bisethylenediamine or -tetrammine series (A = NCS^-)						
1	NO_2^-	[Co$(NH_3)_4$NCS·NO_2]$^+$	62.2	64.0	−1.4	water	Shimura (present paper)
2	NO_2^-	[Co en_2 NCS·NO_2]$^+$	64.8	65.3	−0.5	water	Kuroya and Tsuchida[5]
3	NH_3	[Co en_2 NCS·NH_3]$^{2+}$	60.0	58.8	+1.2	methanol-water	Basolo[3]
4	NCS$^-$	[Co en_2 NCS·NCS]$^+$	59.3	59.0	+0.3	water	Shimura[4], (b)
5	Cl^-	[Co en_2 NCS·Cl]$^+$	59.6	54.0	+5.6	water	Kuroya and Tsuhcida[5], (c)
	(d) Monoammine-bisethylenediamine series (A = NH_3)						
1	NH_3	[Co en_2 NH_3·NH_3]$^{3+}$	63.8	63.8	0.0	water	Basolo[3]
2	H_2O	[Co en_2 NH_3·H_2O]$^{3+}$	61.9	61.9	0.0	water *p*H 0.7	Basolo[3]
3	NCS$^-$	[Co en_2 NH_3·NCS]$^{2+}$	60.0	58.8	+1.2	methanol-water	Basolo[3]
4	OH^-	[Co en_2 NH_3·OH]$^{2+}$	60.6	60.6	0.0	water	Basolo[3]

(a) [Co en_2NH_3·Cl]$^{2+}$ is excluded, because the absorption data[3] of its *trans*-isomer are very queer and not agree with its color.
(b) for the other investigator's data, see Table 1.
(c) for the other investigator's data, see Table 2 a.

specific bands to the *cis-trans* isomerism have thoroughly been clarified, but those of the first and second bands are yet in confusion. It is the purpose of the present paper to report on a new regularity about the first or second band and the geometrical isomerism.

Results and Discussion

First Band.—The first bands of the geometrical isomers of Co(III) complexes available in previous reports as well as in the author's measurements are presented in Tables 1 and 2.

In Table 1, the bands of $[Co\ en_2A_2]$ and $[Co(NH_3)_4A_2]$ type complex ions (A=a molecule or a negative ion) are arranged in the order of the spectrochemical series of the ligand A, *i.e.*,

CN^-, NO_2^-, $SO_3^=$, en, NH_3, ONO^-, H_2O, NCS^-, $SO_4^=$, NO_3^-, OH^-, $C_2O_4^=$, $CO_3^=$, $S_2O_3^=$, Cl^-, $CrO_4^=$, Br^-, I^-.

This represents a decreasing order of the frequencies at the maximum absorption of the first bands of the complexes having such ligands.[2),1)] It is readily recognized from Table 1, that the frequency of the first band of $[Co\ en_2A_2]$ or $[Co(NH_3)_4A_2]$ type ion decreases in the order of the spectrochemical series of the ligand A. From the table, if the data 1b and 6a are omitted, the frequency difference of the first bands of *cis-* and *trans-*isomers, $\nu_c-\nu_t$, is minus for the ligand NO_2^- or $SO_3^=$, zero for NH_3, and plus for H_2O, NCS^- and Cl^-. Considering the very close distribution of NH_3 and en on the spectrochemical series, the fact is generalized as follows. The values $\nu_c-\nu_t$ of $[Co\ X_4A_2]$ type ions are respectively minus or plus according as the position of the ligand A in the spectrochemical series is ahead of or behind the ligand X. This is interpreted as the result of a *trans-*influence of the ligands, that is, the substitution of two A, which is ahead of X on spectrochemical series, for the two X of $[Co\ X_6]$, has a greater hypsochromic effect in the case of *trans-* $[Co\ X_4A_2]$ as compared with the case of the *cis-*$[Co\ X_4A_2]$, while when A is behind X, such substitution has a greater bathochromic effect in the case of *trans-*substitution than the case of *cis*. In summary, it seems that a *trans-*substitution causes a greater shift of the first band than corresponding *cis-*substitution and the direction of this shift is ruled by the relative positions of the ligands on the spectrochemical series. Thus the first bands of *trans-*isomers of dinitro-complexes are at the shorter wave-lengths than the *cis-*isomers, and the reverse relation is observed in the dichloro-complexes.

About $[Co\ X_4A{\cdot}B]$ type complexes, the similar regularities are substantiated from the Table 2. As for monochloro-bisethylenediamine or -tetrammine series, $\nu_c-\nu_t$ is always plus, because Cl^- is far behind en or NH_3 on spectrochemical series. In the mononitro-bisethylenediamine or -tetrammine series, $\nu_c-\nu_t$ is for the most part minus according as the higher position of NO_2^- than that of NH_3 or en. In the monorhodanato-bisethylenediamine or -tetrammine series, the value starts from -1.4 for $[Co(NH_3)_4NCS{\cdot}NO_2]^+$ and comes to $+5.6$ for $[Co\ en_2NCS{\cdot}Cl]^+$. From the data of these three series, it is also shown that the farther separations occur between the ligand B and en or NH_3 on the spectrochemical series, the greater absolute value of $\nu_c-\nu_t$ is attained.

Finally in the monoammine-bisethylenediamine series (Table 2 d), $\nu_c-\nu_t$ is almost zero. This is distinctly due to the condition that the position of NH_3 on the spectrochemical series is very close to that of en and that these complexes are compared to a pentammine type, in which the *cis-trans* isomerism can not be taken place.

Second Band.—The data of the second are summarized in Table 3. Since the second bands of the complexes having NO_2^-, $SO_3^=$ and NCS^-, are generally covered by the large specific bands, the bands of such complexes could not be confirmed. From the table, it is clear that the tendency of $\nu_c-\nu_t$ is the same as that of the first band, though the example of minus

Table 3

Second Band of Geometrical Isomer of Co(III) Complex Ion

Complex ion	Second band (10^{13}/sec.) *cis*-isomer, ν_c	*trans*-isomer, ν_t	$\nu_c-\nu_t$	Ref.
$[Co\ en_2\ NH_3{\cdot}NH_3]^{3+}$	88.8	88.8	0.0	(3)
$[Co\ en_2\ NH_3{\cdot}H_2O]^{3+}$	88.2	87.0	+1.2	(3)
$[Co\ en_2\ H_2O{\cdot}H_2O]^{3+}$	84.5	*ca.* 84.5	*ca.* 0	(3)
$[Co\ en_2\ NH_3{\cdot}OH]^{2+}$	83.3	82.2	+1.1	(3)
$[Co(NH_3)_4H_2O{\cdot}Cl]^{2+}$	82.5	74.5	+8.0	(8)
$[Co\ en_2\ Cl{\cdot}Cl]^+$	*ca.* 76.9	66.7	*ca.* +10.2	(3)

value of $\nu_c - \nu_t$ is unknown.

Moreover, it must be noticed here that some difference values are observed in the case of $[Co\ en_2\ NH_3 \cdot H_2O]^{3+}$ and $[Co\ en_2\ NH_3 \cdot OH]^{2+}$, in which $\nu_c - \nu_t$ of the first band was zero. This indicates that the second band is more sensitive to the geometrical configurations as compared to the first band. Tsuchida[2] has offered a theory concerning the origins of the absorption bands, that the first band is due to the electron transitions in the unsaturated transition shell of the central ion and that the second band is attributed to the transitions of the coördination electrons. The above fact is smoothly explained by this theory of Tsuchida, since the coördination electrons are presumably more sensitive to the geometrical configurations than the electrons in the transition shell of the central ion.

Absorption Spectra and Configurations of Two Isomers of $[Co(NH_3)_4NO_2 \cdot NCS]^+$

The configurations of two stereoisomers of $[Co(NH_3)_4NO_2 \cdot NCS]^+$ have not been studied. The chloride of an isomer was prepared by Werner and Klien[10] from $[Co(NH_3)_4HO_2 \cdot Cl]Cl$, whose structure has been determined as *trans* from its method of preparation[11] and the spectral data.[12] The monohydrated bromide of another isomer was prepared by Werner[13] as the following process.

$$[Co[NH_3)_4(H_2O)_2]_2(SO_4)_3 \xrightarrow{NH_4NCS,\ KBr}$$

$$[Co(NH_3)_4H_2O \cdot NCS]Br_2 \xrightarrow{NaNO_2}$$

$$[Co(NH_3)_4H_2O \cdot NCS](NO_2)_2 \xrightarrow[KBr]{heat}$$

$$[Co(NH_3)_4NO_2 \cdot NCS]Br \cdot N_2O$$

From their preparations, it can be expected that $[Co(NH_3)_4NO_2 \cdot NCS]Cl$ is *trans*-isomer and $[Co(NH_3)_4NO_2 \cdot NCS]Br \cdot H_2O$ is *cis*-isomer. For the purpose of confirming this point of view, the author has prepared the two salts and investigated their absorption spectra, comparing them with those of the corresponding bisethylenediamine salts, which have already been measured by Kuroya and Tsuchida.[5]

The spectrographic measurements were done at room temperature, in the aqueous solutions slightly acidified with acetic acid in order to avoid the decomposition of the salts. The results are shown in Fig. 1 with those of the corresponding bisethylenediamine salts.

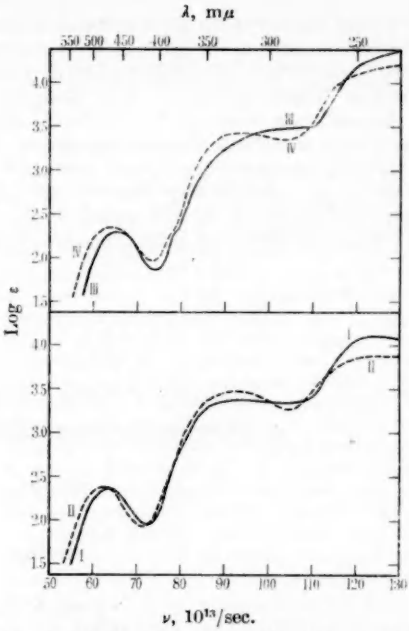

Fig. 1.—Absorption spectra of: I, *trans*-$[Co(NH_3)_4NO_2 \cdot NCS]Cl$; II, *cis*-$[Co(NH_3)_4NO_2 \cdot NCS]Br \cdot H_2O$; III, *trans*-$[Co\ en_2\ NO_2 \cdot NCS]$-NCS (Kuroya and Tsuchida); IV, *cis*-$[Co\ en_2\ NO_2 \cdot NCS]Cl$ (Kuroya and Tsuchida).

As is clear from the shapes of the curves and from the positions of the first bands, $[Co(NH_3)_4NO_2 \cdot NCS]Cl$ is analogous to *trans*-$[Co\ en_2\ NO_2 \cdot NCS]NCS$ and $[Co(NH_3)_4NO_2 \cdot NCS]Br \cdot H_2O$ to *cis*-$[Co\ en_2\ NO_2 \cdot NCS]Cl$. Thus the former is *trans*-salt and the latter *cis*-salt. This conclusion coincides with the above presumption from the method of preparations.

As for the first bands of both salts, the band positions and the logarithms of maximum formal extinction coefficients are as follows;

trans-$[Co(NH_3)_4NO_2 \cdot NCS]Cl$
64.0×10^{13}/sec. ($\log \varepsilon = 2.34$)

cis-$[Co(NH_3)_4NO_2 \cdot NCS]Br \cdot H_2O$
62.6×10^{13}/sec. ($\log \varepsilon = 2.36$).

With regard to the second or third band as well as the nitro- or rhodanato-specific band, it is impossible to explain the detail because of the intricated superposition of these bands.

Summary

A survey of the absorption data of the tetrammine- or bisethylenediamine-Co(III) complexes available in previous literature as well as in the author's measurements has shown that the frequency differences of the first bands

(10) A. Werner and R. Klien, *Z. anorg. Chem.*, **22**, 111 (1900).
(11) S. M. Jörgensen, *ibid.*, **7**, 290 (1894).
(12) R. Tsuchida and S. Kashimoto, This Bulletin, **11**, 785 (1936).
(13) A. Werner, *Ber.*, **40**, 776 (1907).

or second bands) of the *cis*- and *trans*-isomers are closely related to the spectrochemical series of the ligands which coördinated in *cis*- or *trans*-positions. This correlation has been interpreted as the result of a *trans*-influence of the ligands, *i.e.*, a *trans*-substitution causes a greater shift of the first band (or second band) than corresponding *cis*- substitution. The visible and ultraviolet absorption spectra of two isomers of $[Co(NH_3)_4NO_2 \cdot NCS]^+$ have been measured and the steric configurations of both ions have been determined.

The author expresses sincere thanks to Professor R. Tsuchida for his continued interest and encouragement during the course of this work.

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Antituberculous Compounds. I. Synthesis of *p*-Acetamidocinnamaldehyde Thiosemicarbazone

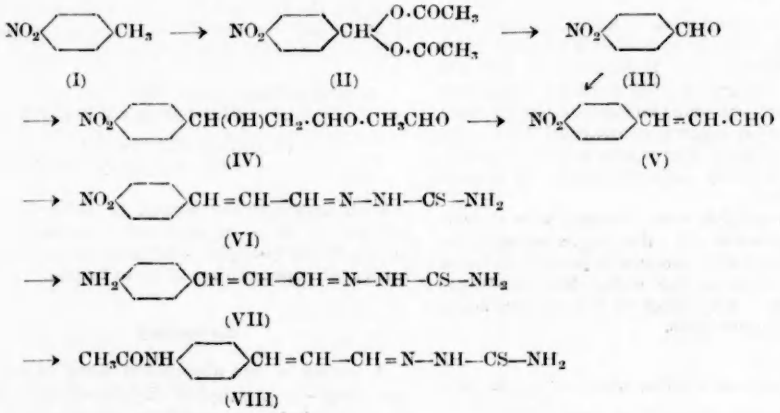
By Tamio NISHIMURA

(Received November 2, 1951)

Some thiosemicarbazones of *p*-substituted aromatic aldehydes have been shown to have the antituberculous activity by Domagk and Behnisch.[1] Among the few thiosemicarbazones active in vivo, *p*-acetamidobenzaldehyde thiosemicarbazone has recently gained much interest and many patients have been treated with this chemotherapeutic agent. The author became interested in preparing *p*-acetamidocinnamaldehyde thiosemicarbazone (VIII) in the expectation that this substance could have antituberculous activity.

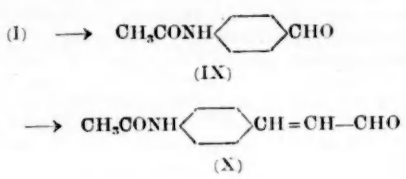
This synthesis of (VIII) may be illustrated by the following series of reactions.

p-Nitrobenzaldiacetate (II) was prepared from *p*-nitrotoluene (I) by the method of Organic Syntheses[2] with certain modifications. The diacetate was easily converted to *p*-nitrobenzaldehyde (III) by hydrolysis. The aldehyde was condensed with acetaldehyde to form β-(*p*-nitrophenyl)-β-hydroxypropionaldehyde acetaldehyde addition compound (IV).[3] The dehydration of the addition compound to form *p*-nitrocinnamaldehyde (V) was accomplished by heating it with hydrochloric acid in acetic acid or methanol. The preparation of (V) by the method of Fecht[4] was also executed and found to give better yields compared with the



(1) G. Domagk, R. Behnisch, F. Mietzsch and H. Schmidt, *Naturwiss.*, **33**, 315 (1946).
(2) "Organic Syntheses," Vol. 18, 61.
(3) C. F. Göhring, *Ber.*, **18**, 372 (1885).
(4) H. Fecht, *Ber.*, **40**, 3898 (1907).

procedure mentioned above. The condensation of (V) with thiosemicarbazide to give *p*-nitrocinnamaldehyde thiosemicarbazone (VI) was performed by the usual procedure. The attempts to reduce (VI) with sodium sulfide or disulfide were unsuccessful; but when sodium hydrogen sulfide was employed, reasonable yields of *p*-aminocinnamaldehyde thiosemicarbazone (VII) were obtained. The reduction with ferrous sulfate also proceeded in ethanol but with lower yields. (VII) was readily acetylated by acetic anhydride in 50% aqueous acetic acid or ethanol to yield *p*-acetamidocinnamaldehyde thiosemicarbazone (VIII). Another approach to (VIII) is illustrated in the following scheme.



p-Acetamidobenzaldehyde (IX) was prepared from *p*-nitrotoluene (I) by refluxing with a solution of sulfur and sodium hydroxide in ethanol followed by acetylation of the ether extract, giving the product melting at 153—155° (yield: 24% of the theoretical.[5],[6] For the preparation of *p*-acetamidocinnamaldehyde Beard's method was followed according to the description in Chemical Abstracts **44**, 8862 (1950) with the following modifications; (a) the product was recrystallized from water, omitting the ether extraction; (b) optimum reaction temperature was found to be −5—10°. From 3.3 g. of *p*-acetamidobenzaldehyde was obtained 0.46 g. (12%) of the desired product melting at 174—175.5°.

Experimental[7]

***p*-Nitrobenzaldiacetate (II).**—To a solution of 50 g. of *p*-nitrotoluene in 400 ml. of acetic anhydride is added 80 ml. of concentrated sulfuric acid.

When the mixture has been cooled to 0°, a solution of 100 g. (1.0 mole) of chromium trioxide in 400 ml. of acetic anhydride[8] is added slowly, with stirring, at such a rate that the temperature does not exceed 10°, and stirring is continued for two hours at 5°—10° in an ice-water bath after the addition is completed. Further treatment is similar to that described in Organic Syntheses. The product, after drying in a vacuum desiccator, weighs 63—69 g. (68—75% of the theoretical), m. p. 122°—124°.

***p*-Nitrocinnamaldehyde (V).**—(a) A mixture of 2.3 grams of β-(*p*-nitrophenyl)-β-hydroxypropionaldehyde acetaldehyde addition compound,[3] 7 ml. of glacial acetic acid and 7 ml. of concentrated hydrochloric acid was heated on a steam-bath. When the aldehyde had dissolved (about one minute), 14 ml. of water were added to the mixture. After cooling, seperated crystals were filtered and washed with water; yield: 17 g. (94%), m. p. 137—140°. (b) Fecht's method with the following modification.—In a 500 ml. three-necked flask fitted with a mechanical stirrer, a thermometer and a burette were placed 50 g. of *p*-nitrobenzaldhyde and 100 ml. of freshly distilled acetaldehyde. The mixture was stirred and cooled in an ice salt mixture. A solution (20%) of potassium hydroxide in methanol was added dropwise to the mixture from the burette until the alkaline reaction was obtained and during the reaction period it was maintained to be alkaline by the additional dropping of the potassium hydroxide solution, amounting 4.8 ml.; the internal temperature was maintained at 0°—5° throughout the procedure, and for 40 minutes thereafter. At the end of this period, the mixture solidified. One hundred and sixty ml. of acetic anhydride were added and heated for 30 minutes on a steam-bath. The solution was poured into 1.2 l. of hot water and heated on a steam-bath for 20 minutes after the addition of 160 ml. of concentrated hydrochloric acid, where the yellow needles separated. The mixture was allowed to stand overnight and crystals were filtered by suction and washed with water. One crystallization from 1.6 l. of 30% acetic acid gave 37.5 g. (64.4%) of the desired product, melting at 140~142°.

***p*-Nitrocinnamaldehyde Thiosemicarbazone (VI).**—To a solution of 18.2 g. of thiosemicarbazide in 700 ml. of hot ethanol was added 35.4 g. of *p*-nitrocinnamaldehyde and the mixture was heated on a steam-bath for 30 minutes. After cooling, yellow precipitates were filtered by suction, washed with ethanol and dried, giving 47 g. (94%) of the desired product, melting at 224° (decomp.). A small sample was recrystallized from ethanol to give the material with the same m. p. (Anal. Calcd. for $C_{10}H_{10}O_2SN_4$: N, 22.38%. Found: N, 22.24%).

(5) Friedl. **4**, 136.
(6) Gabriel and Herzog, *Ber.*, **16**, 2003 (1883).
(7) All temperatures are uncorrected.

(8) Recently, we encountered an explosion on dissolving chromium trioxide in acetic anhydride, the cause of which seems to be local heating of the mixture. Without any hazard, the cooling by tap water has been executed after the total acetic anhydride had been poured in one portion, into the flask containing chromium trioxide but the explosion which we experienced occurred before the cooling was started. Therefore, it is preferable to cool acetic anhydride before being mixed with chromium trioxide.

p-Aminocinnamaldehyde Thiosemicarbazone (VII).—After saturation of a mixture of 200 ml. of ethanol and 64 ml. of 2 *N* aqueous sodium hydroxide solution with hydrogen sulfide, 20 g. of *p*-nitrocinnamaldehyde thiosemicarbazone was added to the mixture in one portion. The mixture was heated on a steam-bath without stirring. When the mixture began to boil, the bath was removed. After the boiling has subsided, the mixture was heated for 10 minutes with stirring and allowed to stand overnight at room temperature. Separated crystals were collected on a filter, washed with ethanol, water and finally ethanol giving 15.5 g. (70 %) of pure product, m. p. 207° (decomp.) (Anal. Calcd. for $C_{10}H_{12}SN_4$: C, 54.53; H, 5.50%. Found: C, 54.29; H, 5.27%).

p-Acetamidocinnamldehyde Thiosemicarbazone (VIII).—(a) To 11 g. of *p*-aminocinnamaldehyde thiosemicarbazone suspended in 110 ml. of ethanol was added 11 ml. of acetic anhydride and the mixture was stirred for ten minutes. After standing overnight, precipitated solid was filtered and washed with water to give 10.4 g. (80 %) of the desired product, m. p. 223—225° (decomp.). Recrystalization from 500 ml. of ethanol yielded 8.4 g. (66 %) of *p*-acetamidocinnamaldehyde thiosemicarbazone, m. p. 224—225° (decomp.). Faint yellow plate was obtained by recrystallization of a small sample from ethanol, m. p. 225° (decomp.) (Anal. Calcd. for $C_{12}H_{14}OSN_4$: C, 54.95; H, 5.38 %. Found: C, 54.89; H, 5.48 %).

(b) Eight-tenths g. of *p*-acetamidocinnamaldehyde was dissolved in 30 ml. ethanol and to the solution 0.4 g. of thiosemicarbazide was added. The mixture was heated on a steam-bath and stirred for 30 minutes while yellow solid precipitated. After cooling, the solid was filtered and washed with ethanol. It weighed 1.1 g. (100 %), m. p. 224° (decomp.). After one recrystallization from ethanol, there was obtained pure material melting at 225° (decomp.).

The author wishes to acknowledge his indebtedness to Miss T. Harada (Agricultural Department, the University of Tokyo) for the microanalytical data.

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Studies on Nitrate Reductase. VI. Acceptor Specificity of the Enzyme

By Ryo SATO,* Mituo EBATA and Fujio EGAMI

(Received October 25, 1951)

Despite a considerable amount of knowledge obtained in our laboratory on nitrate reductase of *Escherichia coli*,[1]~[5] the acceptor specificity of this enzyme remains largely uninvestigated, except a few informations indicating that nitrate reductase of *E. coli* can reduce chlorate as well as nitrate,[1] and that molecular oxygen can not supplant the function of hydrogen acceptor for this enzyme.[6],[7]

Since there are a number of organic nitrogen compounds derived from or structurally related to nitrate, such as nitric acid esters, nitramines, and aliphatic and aromatic nitro compounds, it seems desirable to study whether these compounds can also serve as hydrogen acceptors for nitrate reductase. Among them, nitro compounds are of special interest in view of the long known facts that nitro groups of numerous aromatic compounds are reduced by many microorganisms and animal tissues. Furthermore, in recent years, a considerable attention has been paid to the biochemical significance of nitro groups, since two nitro compounds, chloramphenicol[8] and β-nitropropionic acid[9],[10] have been isolated from biological sources, contrary to the hitherto universally presumed idea that nitro compounds do not occur in nature. It appears, therefore, of interest to find a relation, if exists, between

* Present address : Faculty of Science, Kanazawa University.

(1) F. Egami and R. Sato, *J. Chem. Soc. Japan*, **68**, 39 (1947).

(2) F. Egami and R. Sato, *J. Chem. Soc. Japan*, **69**, 160 (1948).

(3) R. Sato and F. Egami, This Bulletin, **22**, 137(1949).

(4) F. Egami and R. Sato, *J. Chem. Soc. Japan*, **70**, 397 (1949).

(5) R. Sato, *Science Reeuo*, **2**, 122 (1950).

(6) M. Niwa, R. Sato and S. Taniguti, *Symposia on Enzyme Chem.*, **6**, 40 (1951).

(7) L. H. Stickland, *Biochem. J.*, **25**, 605 (1931).

(8) J. Ehrlich, Q. R. Bartz, R. M. Smith, D. A. Joslyn and P. R. Burkholder, *Science*, **106**, 417 (1947).

(9) C. L. Carter and W. J. McChesney, *Nature*, **164**, 575 (1949).

(10) M. T. Bush, O. Touster and J. E. Brockman, *J. Biol. Chem.*, **188**, 685 (1951).

the metabolism of these compounds and nitrate reductase.

The results described below, however, indicate that none of the nitrogen compounds tested can replace nitrate as hydrogen acceptor for nitrate reductase.

Materials and Methods

Dried cells of *E. coli*, which contain nitrate reductase and dehydrogenase systems with their carrier systems, were used as the enzyme preparation. The organism was grown on a peptone-bouillon-agar medium containing 0.1 per cent potassium nitrate for 24 hours at 37°. The cells were then harvested and washed thrice with physiological saline and once with distilled water. The washed cells were dried by lyophilization in the presence of phosphorous pentoxide and stored at 0° in a vacuum desiccator. Dried cell suspension was prepared just before the use by suspending 2—5 mg. of the dried cells in distilled water.

Most experiments were conducted at 37° under an anaerobic condition with the use of Thunberg tubes. Sodium formate was used as the hydrogen donator throughout the investigation. The reaction mixture usually consisted of 1 ml. of each dried cell suspension, $M/10$—$M/25$ sodium formate, substrate solution, $M/5$—$M/15$ phosphate buffer of *p*H 7.4 or 7.6, and distilled water (or inhibitor solution). At the end of incubation periods the reaction mixtures were deproteinized with 2 ml. of saturated uranyl acetate solution or 20 per cent trichloroacetic acid. The reduction of nitrate was followed by determining the formation of nitrite in the deproteinized filtrates. Determination of nitrite was performed by a colorimetric method using Griess-Ilosvay reagent,[11] and analysis for aromatic amines formed from the corresponding nitro compounds was made by the method of Smith and Worrel,[12] N-[1-naphthy]-ethylene diamine being substituted by α-naphthylamine as coupling reagent.

Reoxidation of leuco form of methylene blue by nitrogen compounds was tested as follows. One ml. of each dried cell suspension (3 mg. of the cells), $M/5$ phosphate buffer of *p*H 7.4, $M/25$ sodium formate, and 10^{-4} M methylene blue was pipetted into the main room of a Thunberg tube, and 1 ml. of the solution of the compound to be tested into the side arm. The tube was then incubated at 37° until the dye was completely decolorized. Then the contents of the both parts were mixed. If the compound can reoxidize the leuco dye in the presence of the dried cells, recolorization of the dye takes place immediately after the mixing.

Results

Action of the Dried Cells upon Ethyl Nitrate.

—Ethyl nitrate was used as a representative of nitric acid esters. When the ester is acted by the dried cells of *E. coli*, one may anticipate the following four possibilities: (1) the ester is first hydrolyzed spontaneously or by an esterase to liberate inorganic nitrate, which is in turn reduced to nitrite by nitrate reductase; (2) the ester is enzymatically reduced to the nitrous acid ester; (3) the ester combines with nitrate reductase, but no further change takes place; and (4) no reactions occur at all.

The results of experiments (Curves I and II, in Fig. 1) showed that no nitrite is produced from ethyl nitrate by the action of the dried cells. The hydrolysis of the ester, therefore, seems to be improbable. To test the formation of the nitrous acid ester, the reaction mixture of Curve I was deproteinized after 3 hours incubation and acidified to *p*H 1.0 with hydrochloric acid and then boiled for 30 minutes to hydrolyze the ester. However, no nitrite liberation was detected by this procedure. This seems to exclude the second possibility. Heppel and Hilmoe[13] have reported an enzyme in hog liver which catalyzes the reduction of nitroglycerol and erythritol tetranitrate by glutathione or cysteine in the presence of cyanide. The addition of cysteine (10^{-2} M) and potassium cyanide (4×10^{-3} M), however, did not activate our preparation to reduce ethyl nitrate. It was further found, as is recorded in Fig. 1 (Curves III and IV), that nitrate reduction by the dried cells is entirely

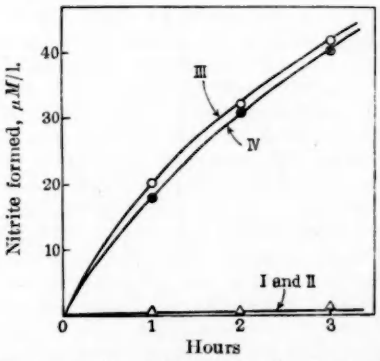


Fig. 1.—Action of the dried cells of *E. coli* on ethyl nitrate (4×10^{-3} M/l.) under aerobic (Curve I) and anaerobic (Curve II) conditions; and reduction of KNO_3 (2×10^{-4} M/l.) by the cells in the presence (Curve III) and absence (Curve IV) of ethyl nitrate (4×10^{-3} M/l.). *p*H = 7.6, and 3 mg. of the dried cells were contained in 5 ml. of medium.

(11) S. Yamagata, *Acta Phytochimica*, **10**, 283 (1938).
(12) G. N. Smith and C. S. Worrel, *Arch. Biochem.*, **24**, 216 (1949).
(13) L. A. Heppel and R. J. Hilmoe, *J. Biol. Chem.*, **183**, 129 (1950).

unaffected by the presence of ethyl nitrate at a concentration 20-fold as much as that of the inorganic nitrate supplied. This fact appears to show that the third possibility is also unlikely. After all, we are compelled to take the fourth possibility and assume that the ester is indifferent to nitrate reductase at all.

That ethyl nitrate has no affinity to nitrate reductase receives further support from the observation that the ester can not reoxidize leuco methylene blue in the presence of the cells, in contrast to the fact that inorganic nitrate can rapidly recolorize the leuco dye under the influence of nitrate reductase.[11]

Effect of Nitrourea on Nitrate Reduction.—Nitrourea, a nitramine, was found to be a strong inhibitory reagent for nitrate reductase. Nitrate reduction by the dried cells was almost completely inhibited by 4×10^{-3} *M* nitrourea. This compound failed to reoxidize leuco methylene blue.

Effect of Nitromethane on Nitrate Reduction.—As a means of elucidating an interaction between nitromethane and nitrate reductase, the effect of this aliphatic nitro compound on the activity of nitrate reductase was studied (Fig. 2). It was found that 4×10^{-3} *M* nitromethane exhibits no inhibition on nitrate reduction by the dried cells, concentrations of nitrate being varied from 2×10^{-5} *M* to 4×10^{-3} *M*. This fact may be thought to indicate that nitromethane has no affinity to nitrate reductase. Furthermore, leuco form of methylene blue was not recolorized by this compound in the presence of the cells.

While rabbit liver homogenates have been reported to decompose nitromethane liberating nitrite ion both under aerobic and anaerobic conditions,[14] the dried cells of *E. coli* produced no nitrite from nitromethane under any conditions.

Reduction of *p*-Nitrobenzoic Acid to *p*-Aminobenzoic Acid.—The dried cells of *E. coli* were found to reduce *p*-nitrobenzoic acid (PNBA) to *p*-aminobenzoic acid (PABA) in the presence of formate. But the amount of PABA produced was very small (Table 1). This reduction was inhibited by 2×10^{-3} *M* cyanide about 80 per cent. Aerobic condition also retarded the reduction, and in the absence of formate the formation of PABA was decreased to 65 per cent of the complete system.

Table 1

The Reduction of PNBA to PABA by the Dried Cells of *E. coli*

Exp. No.	1	2	3	4	5	6
Dried cell suspension (3 mg. per ml.), ml.	1	1	1	1	1*	1
Phosphate buffer (*p*H 7.4, *M*/5), ml.	1	1	1	1	1	1
PNBA (2×10^{-3} *M*), ml.	1	1	1	1	1	—
Sodium formate(*M*/10), ml.	1	1	—	1	1	—
Distilled water, ml.	1	1	2	1	1	3
Condition	an.	an.	an.	ae.	an.	an.
Incubation period, min.	60	120	120	120	120	120
PABA formed (10^{-5} *M*)	0.2	0.3	0.2	0.2	0	0

* The suspension was heated at 100° for 5 minutes. an. = anaerobic; ae. = aerobic.

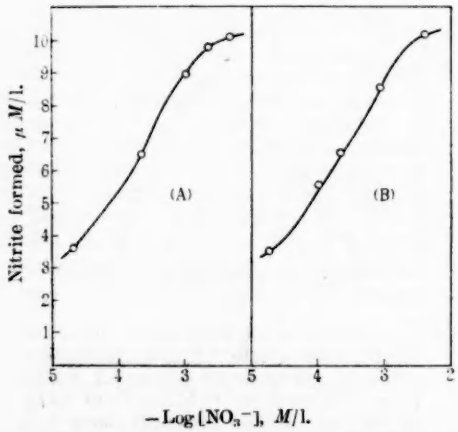


Fig. 2.—Activity-*p*S-curve of nitrate reduction by the dried cells of *E. coli* in the presence (A) and absence (B) of nitromethane (4×10^{-3} *M*/l.). *p*H = 7.6, and 3 mg. of the cells were contained in 5 ml. of medium. Incubation period = 120 minutes.

It was further found that PNBA reduction is also inhibited by nitrate to a small extent. At the first glance this fact was thought to be an evidence indicating that there may be a competitive interaction between PNBA and nitrate. Further study of the effect of PNBA on nitrate reduction, however, provided a strong evidence suggesting that PNBA is indifferent to nitrate reductase. As will be seen in Fig. 3, PNBA (1.4×10^{-3} *M*, a concentration 7-fold as much as the nitrate present) does not inhibit the reduction of nitrate (2×10^{-4} *M*), and, moreover, there is even a slight acceleration in nitrite production. These phenomena can be explained as follows. The

(14) F. Egami and R. Sato, *Nature*, **168**, 365 (1950). F. Egami and M. Itahashi, *J. Biochem.*, **38**, 329 (1951).

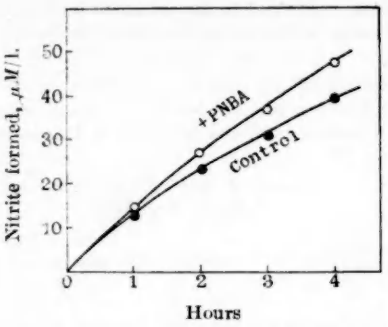


Fig. 3.—Effect of PNBA on nitrate reduction by the dried cells of *E. coli*. pH=7.4, PNBA=1.4×10^{-3} M/l., KNO_3=2×10^{-4} M/l., and 2 mg. of the dried cells were contained in 5 ml. of medium.

enzyme system responsible for PNBA reduction is not identical with that for nitrate reduction, but the observed reduction of PNBA is due to nitrite reductase system contained in the dried cells of *E. coli* at a minute concentration,* and consequently there exists a competitive relation between PNBA reduction and nitrite reduction (but not nitrate reduction). Thus the inhibitory action of nitrate to PNBA reduction mentioned above is not directly caused by nitrate itself, but by nitrite formed from nitrate as the product of its reduction. The slight acceleration of nitrite formation by PNBA is also explicable, since PNBA prevents in a competitive manner the further reduction of nitrite once formed. This competition between nitrite and aromatic nitro groups in their reduction by bacteria has been more clearly demonstrated with a cell-free extract from a bacterial strain, which lacks nitrate reductase, but possesses a much greater activity of nitrite reductase than *E. coli*. We have already published[15] such a competitive interaction between the reduction of the nitro group of chloramphenicol and that of nitrite.

The failure of PNBA to reoxidize leuco methylene blue in the presence of the dried cells of *E. coli* also supports the view that PNBA has no affinity to nitrate reductase.

Several aromatic nitro compounds tested other than PNBA (including chloramphenicol, nitrobenzene, and p-nitrosalicylic acid) were also found to behave similarly.

Discussion

From the evidence presented in this paper it seems very probable that all the nitrogen compounds tested are unable to accept hydrogen from formate by way of nitrate reductase system. Though the compounds tested in the present study are only a few representatives of many related compounds, it might be expected that all the other nitric acid esters, nitramines, and nitro compounds also behave similarly toward nitrate reductase. Thus, based on the results available up to the present, we might be able to state that only two compounds, nitrate and chlorate, can be reduced by this enzyme system. Chlorate has been known to be reduced to chlorite by *E. coli* and some other bacteria.[16] The ability of this compound to accept hydrogen from nitrate reductase was concluded by us[1] from the observations that chlorate, which may be regarded as a chlorine analog of nitrate, exihibts a marked competitive inhibition on nitrate reduction by a cell-free preparation of nitrate reductase from *E. coli*. We have further found, by analyzing the inhibition data, that the apparant Michaelis constant for chlorate reduction by this enzyme is $10^{-2.98}$ M/l., while that for nitrate reduction is $10^{-3.85}$ M/l. Chlorate reduction, however, could not be regarded as a normal function of this enzyme, since its reduction product, chlorite, has been shown to be a very powerful bacterial poison.[16] Quastel, Stephenson, and Whetham[16] claimed that for chlorate reduction is responsible an enzyme which is similar but distinct from nitrate reductase. It might be quite natural that Stephenson[17] was led by this opinion to a somewhat queer conclusion, which she wrote as follows: "Chlorate is a salt which so seldom forms a part of bacterial substrate that it can hardly worth while to possess an enzyme for using it.... the (chlorate reducing) enzyme can hardly be of service to the cell except for purposes of suicidal war". On the other hand, according to our demonstration of the identity of the chlorate reducing enzyme with nitrate reductase, such a queer consideration is not necessary; chlorate reduction is a mere result of a chemical resemblance of chlorate to nitrate, which permits the former to act as hydrogen acceptor for nitrate reductase.

* The content of nitrite reductase in *E. coli* varies from a strain to the other. One strain is completely devoid of it, while the other contains a relatively high activity. But our experiences indicate that the cell-free preparations of nitrate reductase extracted from many strains of *E. coli* by ultrasonic destruction of the cells are free from nitrite reductase activity.

(15) F. Egami, M. Ebata and R. Sato, *Nature*, **167**, 118 (1951).

(16) J. H. Quastel, M. Stephenson and M. D. Whetham, *Biochem. J.*, **19**, 304 (1925).

(17) M. Stephenson, in "Perspectives in Biochemistry", Cambridge, 1938, p. 92.

Summary

Evidence is presented to indicate that ethyl nitrate, nitromethane, and possibly also nitrourea are not able to accept hydrogen from nitrate reductase system of *E. coli*. *p*-Nitrobenzoic acid is reduced to *p*-aminobenzoic acid by the action of the dried cells of *E. coli* to a small extent. This reduction is, however, not achieved by nitrate reductase. Nitrite reductase contained in the organism is responsible for the reduction of aromatic nitro groups.

The cost of this research has been defrayed from the Grant in Aid for Fundamental Scientific Research from the Ministry of Education.

Chemical Institute, Faculty of Science, Nagoya University

On the Hydrogen-Bond-Forming Powers of Atoms or Atomic Groups

By Masamichi TSUBOI

(Received November 10, 1951)

I. Introduction

It is well known that the energy of hydrogen bond, X—H···Y where X and Y denote two kinds of atoms, takes various values (from about 8 to 1 kcal./mol) depending upon the nature of the atomic groups which contain X and Y. However, as to how the energy relates with the nature of the atomic groups much is yet to be studied. Useful data concerning this question may be obtained from infrared absorption measurements of certain ternary solutions—the ternary solutions, each of which is composed of a large quantity of nonpolar solvent (such as CCl_4) and small quantities of two polar solutes (a proton-donator containing X—H and a proton-acceptor containing Y), and in which complex molecules with the hydrogen bond X—H···Y are present.* For, from these measurements the concentrations of hydrogen-bonded X—H and free X—H in the solutions at various temperatures are to be found, and on the basis of such data the bond energy of any X—H···Y can be determined. The difference in the values of energies thus obtained for different hydrogen bonds in the same medium must represent the difference in the nature of the atomic groups pertaining to the bonds, there being no difference in the environment in which the hydrogen bonds are formed.

This paper is concerned with preliminary investigations made with what have been stated above in mind. In the first place, some results of the infrared absorption measurements of certain ternary solutions are presented, with interpretations of them. Next, it is shown how the hydrogen bond energy can be determined from the results of these measurements, with a few examples of the values of the energy actually obtained. Lastly, the general relationship between the hydrogen-bond-forming powers of atoms and the nature of the atomic groups containing the atoms is discussed, on the basis of the data so far obtained.

* Of this idea the writer owes to Dr. T. Shimanouchi's suggestion.

II. Instrument and Samples

The instrument used is a reflection monochromator with a 60° prism of fluorite,(1) in conjunction with the thermocouple and galvanometer system.

The compositions of the ternary solutions examined are:(2)

phenol	+ ethyl ether	+ carbon tetrachloride
〃	+hexamethylene tetramine	+ 〃
〃	+ benzaldehyde	+ 〃
〃	+ anisol	+ 〃
〃	+ nitrobenzene	+ 〃
〃	+methyl benzoate	+ 〃
methanol	+ethyl ether	+ 〃
〃	+ anisol	+ 〃
guaiacol	+hexamethylene tetramine	+ 〃
o-nitrophenol	+ 〃	+ 〃
methyl salicylate	+ 〃	+ 〃
salicylic aldehyde	+ 〃	+ 〃
p-oxybenzoic methylester	+ 〃	+ 〃

(1) The fluorite prism used was kindly placed by O. Matsumura at the writer's disposal.

(2) Some of the materials used were kindly placed at the writer's disposal by Prof. S. Kambara, Mr. H. Tada, Mr. H. Baba, and Mr. T. Inukai.

For comparison, infrared absorption measurements were made also of the binary solutions with the following compositions:

phenol	+	carbon tetrachloride
methanol	+	〃
guaiacol	+	〃
o-nitrophenol	+	〃
methyl salicylate	+	〃
salicylic aldehyde	+	〃
ethyl ether	+	〃
hexamethylene tetramine	+	〃
benzaldehyde	+	〃
anisol	+	〃
nitrobenzene	+	〃
methyl benzoate	+	〃

III. Infrared Absorption Data and their Interpretations

Formation of Intermolecularly Hydrogen-bonded Complexes in Certain Ternary Solution.—Figs. 1—10 show the results of infrared absorption measurements of phenol and methanol in various solutions. In these figures the abscissas denote wave-lengths in the 3μ region and the ordinates the absorption coefficients of phenol or methanol. These absorption coefficients were obtained by subtraction: the absorption coefficients of the solutions containing phenol or methanol [*e. g.* solution composed of phenol (0.01 mol/l.)+ethyl ether (0.06 mol/l.)+carbon tetrachloride] minus the absorption coefficients of the solutions without phenol or methanol [*e. g.* solution composed of ethyl ether (0.06 mol/l.)+carbon tetrachloride].

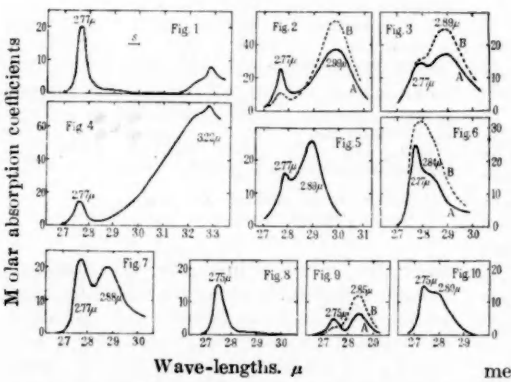


Fig. 1–10.—Infrared absorption curves of phenol and those of methanol in the solutions: Fig. 1, CCl_4+phenol 0.01 mol/l.; Fig. 2, (A) CCl_4+phenol 0.01 mol/l.+ethyl ether 0.06 mol/l., (B) CCl_4+phenol 0.01 mol/l.+ethyl ether 0.11 mol/l.; Fig. 3, (A) CCl_4+phenol 0.01 mol/l.+benzaldehyde 0.05 mol/l., (B) CCl_4+phenol 0.01 mol/l.+benzaldehyde 0.1 mol/l.; Fig. 4, CCl_4+phenol 0.01 mol/l.+hexamethylene tetramine 0.01 mol/l.; Fig. 5, CCl_4+phenol 0.01 mol/l.+anisol 0.5 mol/l.; Fig. 6, (A) CCl_4+phenol 0.01 mol/l.+nitrobenzene 0.08 mol/l., (B) CCl_4+phenol 0.01 mol/l.+nitrobenzene 0.18 mol/l.; Fig. 7, CCl_4+phenol 0.01 mol/l.+methyl benzoate 0.02 mol/l.; Fig. 8, CCl_4+methanol 0.02 mol/l.; Fig. 9, (A)CCl_4+methanol 0.01mol/l.+ethyl ether 0.1 mol/l., (B) CCl_4+methanol 0.01 mol/l.+ethyl ether 0.4 mol/l.; Fig. 10, CCl_4+methanol 0.02 mol/l.+anisol 0.5 mol/l. Temperature: 30°C. *s*: Effective slit-width.

The curve in Fig. 1 is for phenol in the solution of the composition: phenol (0.01mol/l.)+carbon tetrachloride. There is seen a single O—H band at 2.77μ. This indicates that the phenol molecules here are wholly in a free state without molecular association.

Figs. 2—7 show how the absorption is effected by adding proton-acceptors to the solution. For example, in Fig. 2, two curves A and B are for phenol in the ternary solutions, respectively with compositions: phenol (0.01 mol/l.)+ ethyl ether (0.06 mol/l.)+carbon tetrachloride and phenol (0.01 mol/l.)+ ethyl ether (0.11 mol/l.)+carbon tetrachloride. Here, two O—H bands are present, one at 2.77 μ and the other at 2.99 μ. A comparison of the three curves, that in Fig. 1 and A and B in Fig. 2, reveals that by adding ethyl ether to the solution, phenol (0.01 mol/l.)+carbon tetrachloride, the absorption intensity of the O—H band at 2.77 μ becomes weaker and there appears another O—H band at 2.99 μ. Further experiments have shown that, on increasing the concentration of ethyl ether in the solution, there occurs a decrease in the absorption intensity of 2.77 μ band and an increase in the intensity of 2.99 μ band, but no shifting occurs in the positions of these O—H bands. These facts, taken together, may be interpreted as indicating that in the above-mentioned solutions a molecular complex: $C_6H_5OH\cdots O(C_2H_5)_2$, to which the 2.99 μ band is attributable, is formed by the intermolecular hydrogen bond O—H···O, and that this complex molecule coexists with free phenol and ether molecules in equilibrium: $C_6H_5OH+O(C_2H_5)_2 \rightleftarrows C_6H_5OH\cdots O(C_2H_5)_2$. The curves in Figs. 3—7 are for phenol in the ternary solutions, whose compositions are given in the notes under the figures. Each of these solutions contains, besides phenol, proton-acceptor. In these solutions phenol exhibits O—H bands at 2.89 μ, 3.22 μ, 2.89 μ, 2.84 μ, and 2.88 μ, besides that at 2.77 μ, indicating

that there are formed in the solutions complexes: $C_6H_5OH\cdots OCHC_6H_5$, $C_6H_5OH\cdots N_4(CH_2)_6$, $C_6H_5OH\cdots O(CH_3)C_6H_5$, $C_6H_5OH\cdots O_2NC_6H_5$, and $C_6H_5OH\cdots OC(OCH_3)C_6H_5$[3].

Figs. 8, 9, and 10 give data of the solutions in which methanol is present, instead of phenol, as a proton-donator. The curves here (for methanol) are much like those in Figs. 1, 2, and 5 (for phenol), and similar interpretations may be given of these data. Thus, Fig. 9 shows that in the ternary solution with the composition: methanol (0.01 mol/l.) + ethyl ether (0.1 or 0.4 mol/l.) + carbon tetrachloride, there is formed a molecular complex: $CH_3OH\cdots O(C_2H_5)_2$, to which the 2.85 μ band is attributable; and Fig. 10 shows that in solution with the composition: methanol (0.02 mol/l.) + anisol (0.5 mol/l.) + carbon tetrachloride, there is formed a molecular complex: $CH_3OH\cdots OCH_3C_6H_5$, to which the 2.80 μ band is attributable.

Summarizing, it may be said that in a carbon tetrachloride solution containing small quantities of a proton-donator (D) and a proton-acceptor (A), there is formed a molecular complex DA, where D and A are intermolecularly hydrogen-bonded, and that the complex DA, coexists with free D and free A in equilibrium: $D+A \rightleftarrows DA$.

Hindrance of the Presence of *Intra*-molecular Hydrogen Bond to the Formation of *Inter*-molecular Hydrogen Bond.— What has been said in the preceding section is of phenol or methanol without *intra*-molecular hydrogen bond; however another relation is revealed of *o*-substituted phenols with *intra*-molecular hydrogen bond. The data obtained from the infrared absorption measurements, as to the effects of adding proton-acceptor to dilute carbon tetrachloride solutions of some substituted phenols are given in Table 1. All the phenols here examined, excepting *p*-oxybenzoic methylester, have O—H's in intramolecularly hydrogen-bonded state; and the positions and the intensities of the O—H bands of these phenols in their dilute carbon tetrachloride solutions remain unchanged by adding hexamethylene tetramine (a powerful proton-acceptor) to the solutions. *p*-Oxybenzoic methylester, with no *intra*-molecular hydrogen bond, exhibits a different behavior. There, on adding hexamethylene tetramine to its dilute carbon tetrachloride solution, a new O—H band appears at 3.22 μ, besides the free O—H band (at 2.77 μ), just like nonsubstituted phenol. The facts as above may be interpreted as indicating that the presence of the *intra*-molecular hydrogen bond, even such a weak one as in guaiacol, hinders the OH group therein involved from forming an *inter*-molecular hydrogen bond.

Table 1

Composition of solution	Concentration, mol/l.	Wave-length (in μ) of O—H band; with molar absorption coefficient in parentheses
Guaiacol Carbon tetrachloride	0.005	2.80 (80)
Guaiacol Hexamethylene tetramine Carbon tetrachloride	0.005 0.03	2.80 (80)
o-Nitrophenol Carbon tetrachloride	0.005	3.08 (80)
o-Nitrophenol Hexamethylene tetramine Carbon tetrachloride	0.005 0.03	3.08 (80)
Methyl salicylate Carbon tetrachloride	0.005	3.11 (120)
Methyl salicylate Hexamethylene tetramine Carbon tetrachloride	0.005 0.03	3.11 (120)
Salicylic aldehyde Carbon tetrachloride	0.005	3.1
Salicylic aldehyde Hexamethylene tetramine Carbon tetrachloride	0.005 0.03	3.1
p-Oxybenzoic methylester Hexamethylene tetramine Carbon tetrachloride	0.005 0.03	2.77 (20) 3.22 (120)

Effective slit width: 0.025 μ
Temperature: 10°C.

IV. On the Determination of the Intermolecular Hydrogen Bond Energy

The infrared absorption data of the solutions as those given above are useful for the determination of the energies of *inter*-molecular hydrogen bonds between proton-donating and proton-accepting molecules. From these data the values of energies are obtainable in the way as follows.

For the ternary solutions ($D+A+CCl_4$) with such low concentrations of D and A as those examined here, it is admissible to consider that there are present only the complex molecules DA and the free molecules, D and A, but none of other complex molecules such as D_2, D_2A, etc. Then the total concentrations

(3) As a result of the electron migration from the methoxyl O to the carbonyl group in the methyl benzoate molecule, the carbonyl O in this molecule is expected to have a stronger negative charge, and must therefore be a stronger proton-acceptor than the methoxyl O.

of D and of A in the solutions may be taken as equal respectively to the sum of the concentrations of DA and D, and to the sum of the concentrations of DA and A, *i. e.*,

$$\text{Total concentration of D} = (\text{DA}) + (\text{D})$$

$$\text{and Total concentration of A} = (\text{DA}) + (\text{A}),$$

where (DA), (D), and (A) denote the concentrations (mol/l.) of DA, D, and A, respectively.

Now, from the intensity of the absorption band due to DA or free D and the known total concentrations of D and A, is obtainable the value of the equilibrium constant:

$$K = \frac{(\text{DA})}{(\text{D}) \times (\text{A})} \qquad (1)$$

for $\text{D} + \text{A} \rightleftarrows \text{DA}$. In order to facilitate the finding of the values of K, Fig. 11 is constructed, where are given the relation between (DA)+(A) and

$$\alpha = \frac{(\text{DA})}{(\text{D}) + (\text{DA})} \qquad (2)$$

(which represents the degree of forming the complex DA for D), for various values of K and for the fixed concentration, 0.01 mol/l., of (D)+(DA).

The change of free energy (ΔF) accompanying the formation of DA is obtainable from K by the equation: $\Delta F = -RT \ln K$, and then the change of total energy (ΔE) and the change of entropy (ΔS) accompanying the formation of DA (in carbon tetrachloride medium) are obtainable from ΔF for different temperatures by the equation: $\Delta F = \Delta E - T\Delta S$.

Two examles of the actual determinations of the energies of *inter*-molecular hydrogen bonds between D and A in the carbon tetrachloride solutions are given below:

ΔE of the Hydrogen Bond between Phenol and Ethyl Ether.—A determination was made of the O—H···O bond energy from the intensity measurements of the 2.99 μ band which is due to the molecular complex: $C_6H_5OH\cdots O(C_2H_5)_2$ (*cf.* Fig. 2). By preliminary examinations, it has been ascertained that the relation between the observed transmittance and the absorption path-lengths of the solutions (phenol+ethyl ether+CCl_4) for the 2.99 μ ray accords with the Lambert's law, at any concentrations and temperatures (30—60°C.). Moreover, it has been ascertained that on the 2.99 μ band there is no superposition of other bands. These facts show that the observed molar absorption coefficient (κ) at 2.99 μ of phenol in these solutions may be taken as being proportional to α, thus

$$\kappa = \kappa_0 \alpha \qquad (3)$$

Here, the proportionality constant κ_0 corresponds to the maximum value of κ, *i. e.*, the molar absorption coefficient of the molecular complex: $C_6H_5OH\cdots O(C_2H_5)_2$, at 2.99 μ. The values of κ was observed of the solutions of various concentrations of ethyl ether and of constant concentration of phenol, 0.01 mol/l., and are given by ○ (at 30°C.), △ (at 45°C.), and □ (at 60°C.), in Fig. 12. The dotted lines in Fig. 12. represent the values of κ calculated from the equations (1), (2), and (3) by giving

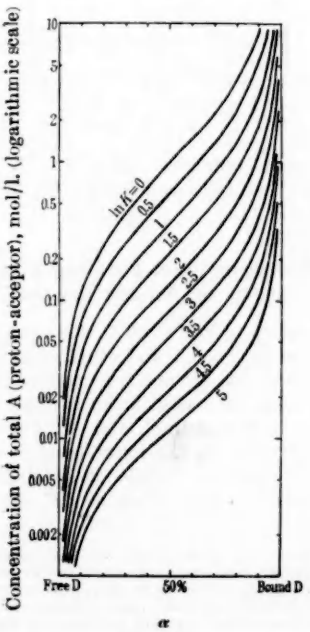


Fig. 11.

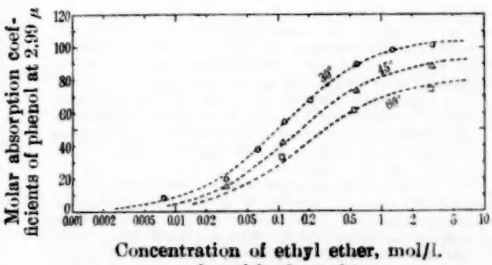


Fig. 12.

following values to κ_0 and K in them: $\kappa_0=105$, $K=10.0$ at 30°C.; $\kappa_0=94$, $K=7.4$ at 45°C.; $\kappa_0=82$, $K=5.75$ at 60°C.[4] (also *cf.* Fig. 11). As is seen in the figure, the observed and calculated values of κ are in close agreement with each other, and hence, from the values of K given here the values of ΔF, ΔS, and ΔE for the O—H···O bond formation can be calculated. These are tabulated below.

Table 2

Temperature, °C.	K	ΔF, kcal./mol	ΔS, E. U.	ΔE, kcal./mol
30	10.0	−1.38		
45	7.4	−1.26	−7.6	−3.7
60	5.75	−1.16		

ΔF (at 30°C.) of the Hydrogen Bond between Phenol and Hexamethylene Tetramine.—A determination was made of the ΔF (at 30°C.) of the O—H···N bond from the intensity measurement of the 2.77 μ band which is due to free phenol molecules (*cf.* Fig. 4). As a preliminary examination the transmittance of the 2.77 μ ray was measured in binary solutions composed of phenol+carbon tetrachloride, varying the concentration of phenol from 0 to 0.006 mol/l. but keeping the absorption path-length (6.11 cm.) and the temperature (30°C.) of the solution constant;[5] and then, similar measurements were made with the ternary solutions which contain hexamethylene tetramine of 0.03 mol/l. The results obtained are shown in Fig. 13. From this figure the concentrations of the phenol molecules in a free state and of those bound by the hexamethylene tetramine molecules were read. In Fig. 14, the ratio of the total concentration of hexamethylene tetramine to the concentration of bound phenol in each solution is plotted versus the reciprocal of the concentration of the free phenol. Here, all the plotted points fall approximately on a single straight line. From the inclination of this straight line, K and ΔF for the O—H···N bond formation at 30°C. were obtained after I. M. Klotz's method,[6] with the results: $K=40$, $\Delta F=-2.4$ kcal./mol.

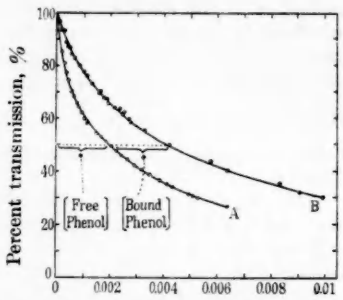


Fig. 13.—Percent transmittance of phenol in (A) binary solutions, CCl_4+phenol, and (B) ternary solutions, CCl_4 + phenol + hexamethylene tetramine (0.03 mol/l.). Wavelength: 2.77 μ. Cell thickness: 6.11 cm. Temperature: 30°C.

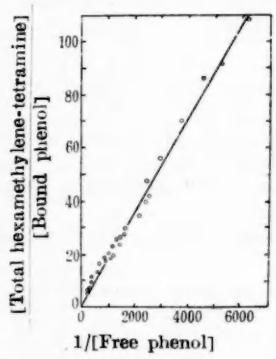


Fig. 14.

V. Hydrogen-Bond-Forming Powers of Atoms or Atomic Groups

The values of ΔF at 30°C. for the hydrogen bond between phenol and ethyl ether and for that between phenol and hexamethylene tetramine are, as have been given in the preceding section, −1.38 kcal./mol, and −2.4 kcal./mol, respectively;—*i. e.*, the latter bond is stronger than the former. While it is difficult to determine the exact values of ΔF and ΔE for

(4) On raising the temperature from 30 to 60°C., the position of the maximum absorption in the band due to the molecular complex shifts slightly (about 0.02 μ) to a shorter wave-length, and κ_0 decreases from 105 to 82. This fact shows that the structure of the molecular complex concerned is not strictly the same at different temperatures. In the present investigation, which aims at approximate estimation of ΔE, this difference in structure has been ignored.

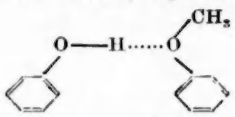
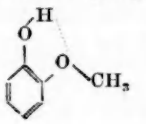
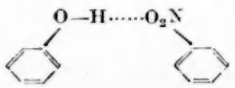
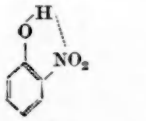
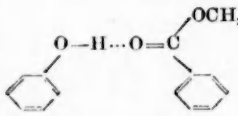
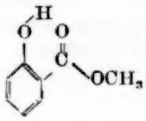
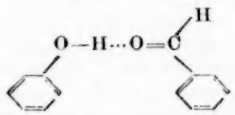
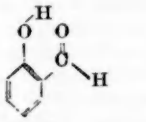
(5) This preliminary examination was necessary because the observed absorption at 2.77 μ of phenol in carbon tetrachloride solution, even when its concentration is as low as 0—0.01 mol/l., does not obey Beer's law. This disobedience is but apparent, due to too low resolving power of the monochromator used (effective slit width: 0.05 μ) for the sharpness of the selective absorption of phenol at 2.77 μ.

(6) I. M. Klotz, F. M. Walker, and R. B. Pivan, *J. Am. Chem. Soc.*, **68**, 1486 (1946). Because of the low solubility of hexamethylene tetramine in CCl_4, it is impossible to use Klotz's method in determining the maximum number of phenol molecules that can be bound by one hexamethylene tetramine molecule. (Probably the maximum number is 4.)

the intermolecular hydrogen bonds in the other complexes examined, the order of the strengths of these hydrogen bonds in these complexes may be determined by observing the frequencies and the intensities of the O—H bands concerning the hydrogen bonds in question. Generally speaking, the frequency and the intensity of the hydrogen-bonded O—H band are taken as measures of the strength of the bond, if other conditions are equal. For, the lower frequency of the bonded O—H band indicates the greater loosening of the O—H accompanying the hydrogen bond formation, and the larger intensity indicates the higher concentration of the hydrogen-bonded O-H and accordingly indicates the larger value of K for the formation of the hydrogen-bonded complex. Inspections of Figs. 2, 5, 9, and 10, with what have been just stated in mind, reveal that the O—H···O bonds in the complexes: phenol-ethyl ether and phenol-anisol are stronger respectively than the O—H···O bonds in the complexes: methanol-ethyl ether and methanol-anisol, and that the OH of phenol is stronger than the OH of methanol in the tendency of donating its proton on its forming hydrogen bonds with other molecules. Similarly, from inspections of Fig. 2—7 and 9—10 it may be concluded that the proton-accepting powers of atoms in the molecules examined are in the following order, from stronger to weaker: N of hexamethylene tetramine, O of ethyl ether, O of benzaldehyde, O (carbonyl) of methyl benzoate, O of anisol, O of nitrobenzene.

From the order of the proton-donating and proton-accepting powers of the atoms and atomic groups as exemplified in the above, the following general rule may be advanced: The proton-donating power of X—H depends upon the tendency with which X attracts the electron of H to leave the proton bare; and the proton-accepting power of Y depends upon the tendency with which Y attracts electrons from the adjacent atoms or adjacent atomic groups to charge itself electrically negative. This rule agrees with and adds to what L. Pauling said concerning the strength of the hydrogen bond: "The strength of the hydrogen bond should increase with the increase in the electronegativity of the two bonded atoms (X and Y)."[7] The validity of the rule here advanced may be demonstrated also from the behavior of the O atom attached to the benzene ring. Thus, the electron migration in phenol molecule from O to benzene ring[8] results in an increase of the tendency of the H in OH to be left bare, and accordingly in increase of the proton-donating power of the OH group; this electron migration, on the other hand, results in a decrease of the negativity of the O atom attached to the benzene ring, and accordingly in the decrease of its proton-accepting power. (This last effect is apparent

Table 3

Inter-molecular hydrogen bonds	ν_{OH}, cm.$^{-1}$	$\Delta\nu_{OH}$, cm.$^{-1}$	*Intra*-molecular hydrogen bonds	ν_{OH}, cm.$^{-1}$	$\Delta\nu_{OH}$, cm.$^{-1}$
	3460	150		3560	50
	3520	90		3250	360
	3470	140		3220	390
	3460	150		~3200	~400

(7) L. Pauling: The Nature of the Chemical Bond," 287 (1940). The words in the parentheses are the present writer's.

(8) K. F. Herzfeld: *Chem. Rev.*, **41**, 233 (1947); S. Nagakura and H. Baba: *J. Chem. Soc. Japan*, **71**, 527 (1950).

from the fact that the O atom in anisol has less proton-accepting power than that in ethyl ether.—*Cf.* the order in proton-accepting powers given above.)

Appendix.—In the course of the present investigation, an interesting fact has come to the writer's notice, *viz.*—The frequency of O—H, bonded with a certain atomic group, takes different values according as the bond is *intra*-molecular or *inter*-molecular. Table 3 gives the values of ν_{OH} (the frequency of O—H stretching vibration) and $\Delta\nu_{OH}$ (its difference from the frequency, 3610 cm^{-1}, of free O—H stretching vibration) given for some molecules and molecular complexes with *intra*-molecular and *inter*-molecular hydrogen bonds.

As is shown in the table, ν_{OH} in guaiacol is higher than that in the complex: $C_6H_5OH\cdots OCH_3C_6H_5$. This fact may be explained from the difference in the structural positions of oxygen atoms in these compounds; for, in guaiacol the oxygen atom of the methoxyl group which is the proton-acceptor, is situated at a position much displaced from the extention of the line representing the O—H bond vector. ν_{OH}'s for the other three O—H···O bonds listed in the table take lower values when the bonds are formed *intra*-molecularly than when they are formed *inter*-molecularly. This may be explained by assuming that there occurs a conjugation across the hydrogen bonds[9] when the bonds are formed *intra*-molecularly in *o*-nitrophenol, methyl salicylate, and salicylic aldehyde.

The writer wishes to express his sincere thanks to Professor San-ichiro Mizushima and Dr. Takehiko Shimanouchi for their kind guidance throughout this work. His thanks are also due to O. Matsumura for his lending the fluorite prism used, and to Professor S. Kambara, Mr. H. Tada, Mr. H. Baba, and Mr. T. Inukai for their placing some of the materials used at the writer's disposal. The cost of this research has been partly defrayed from Grant in Aid for Scientific Research from the Ministry of Education.

(9) R. S. Rasmussen, D. D. Tunnichiff, and R. Brattain, *J. Am. Chem. Soc.*, **71**, 1068 (1949); R. S. Rasmussen and R. Brattain, *ibid.*, 1073; I. M. Hunsberger, *J. Am. Chem. Soc.*, **72**, 5626 (1950).

1,2-Bis-(*p*-methoxyphenyl)-1-(methylthio)-butane, 1,2-Bis-(*p*-methoxyphenyl)-1-(methylthio)-2-methoxy-ethane, and 1,2-Bis-(*p*-methoxyphenyl)-1,2-bis-(methylthio)-ethylene. Estrogenic Sulfur Analogs of Hexestrol Dimethyl Ether and of Diethylstilbestrol Dimethyl Ether

By Yoshiyuki URUSHIBARA, Michinori ŌKI, and Reiji IKEDA

(Received November 12, 1951)

The syntheses of some estrogenic analogs of hexestrol have already been reported from this laboratory.[1][2] In the present paper the syntheses of further analogs of hexestrol dimethyl ether and of diethylstilbestrol dimethyl ether are described.

1,2-Bis-(*p*-methoxyphenyl)-1-(methylthio)-butane, $CH_3OC_6H_4CH(SCH_3)CH(C_2H_5)C_6H_4OCH_3$.—**1,2-Bis-(*p*-methoxyphenyl)-butanol-(1),** $CH_3OC_6H_4CHOHCH(C_2H_5)C_6H_4OCH_3$, m. p. 100-103°, was prepared by the reduction of the corresponding ketone, α-ethyldesoxyanisoin, with sodium amalgam in alcoholic solution.[3] The substituted butanol (1.0 g.) and methyl mercaptan (2 cc.) were dissolved in glacial acetic acid (30 cc.) and dry hydrogen chloride was passed into the solution for an hour, methyl mercaptan (1 cc.) being added every fifteen minutes. After the addition of a small amount of zinc chloride, the mixture was left to stand overnight under a stopper. The crystalline deposits were collected and recrystallized from ethyl acetate. 1,2-Bis-(*p*-methoxyphenyl)-1-(methylthio)-butane (0.55 g.) was obtained in colorless prisms melting at 152.5-154.5° and soluble with difficulty in alcohol.

(1) Y. Urushibara and M. Ōki, This Bulletin, **23**, 35 (1950).
(2) Y. Urushibara and T. Takahashi, *ibid.*, **23**, 53 (1950).
(3) T. Takahashi, read before the annual meeting of the Chemical Society of Japan on April 2, 1950.

Yield: 49% of the theory. Found: S, 9.95%; molecular weight, 329. Calculated for $C_{19}H_{24}O_2S$: S, 10.13%; molecular weight, 316.

1, 2-Bis-(*p*-methoxyphenyl)-1-(methylthio)-2-methoxy-ethane, $CH_3OC_6H_4CH(SCH_3)CH(OCH_3)C_6H_4OCH_3$.—Anisoin methyl ether, $CH_3OC_6H_4CH(OCH_3)COC_6H_4OCH_3$, prepared by the action of methyl iodide and silver oxide on anisoin was reduced to hydranisoin methyl ether, $CH_3OC_6H_4CH(OCH_3)CHOHC_6H_4OCH_3$, with sodium amalgam.[4] The hydranisoin methyl ether (1.0 g.) was treated with methyl mercaptan and hydrogen chloride in the same way as described above. The product was recrystallized from ethyl acetate or alcohol. 1,2-Bis(*p*-methoxyphenyl)-1-(methylthio)-2-methoxyethane was obtained in colorless prisms melting at 190-191°, soluble in glacial acetic acid and in ethyl acetate, and soluble with difficulty in alcohol and in acetone. Yield: 55% of the theory (0.6 g.). Found: S, 10.25%; molecular weight, 305. Calculated for $C_{18}H_{22}O_3S$: S, 10.07%; molecular weight, 318.

Attempted preparation of anisoin methyl ether from α-bromodesoxyanisoin, $CH_3OC_6H_4CHBrCOC_6H_4OCH_3$, failed. The latter compound was prepared by the bromination of desoxyanisoin in direct sun light. α-Bromodesoxyanisoin melts at 101-103° with decomposition. Found: Br, 23.83%. Calculated for $C_{16}H_{15}O_3Br$: Br, 23.84%. It gave anisoin by the action of either sodium methoxide or sodium ethoxide.

1,2-Bis-(*p*-methoxyphenyl)-1,2-bis-(methylthio)-ethylene, $CH_3OC_6H_4C(SCH_3)=C(SCH_3)C_6H_4OCH_3$.—Anisoin (0.6 g.) was dissolved in glacial acetic acid (30 cc.). Methyl mercaptan (1 cc.) was added to the solution and dry hydrogen chloride was passed for an hour, while methyl mercaptan (0.5 cc.) was added every fifteen minutes. After the addition of a small amount of zinc chloride, the mixture was left to stand overnight. Deposited crystals were collected and recrystallized from ethyl acetate. 1,2-Bis-(*p*-methoxyphenyl)-1,2-bis-(methylthio)-ethylene was obtained in colorless prisms melting at 194-196° and soluble with difficulty in alcohol. Analysis gave the required result. Found: C, 65.65; H, 6.19; S, 19.12%; molecular weight, 337. Calculated for $C_{18}H_{20}O_2S_2$: C, 65.02; H, 6.06; S, 19.29%; molecular weight, 332. $C_{18}H_{22}O_2S_2$ would require H, 6.72%. The absorption spectrum of this substance gave a maximum at 235 mμ coinciding with the maximum given by diethylstilbestrol dimethyl ether, showing the presence of a similar dimethoxy-stilbene structure. Furthermore, it was different from the saturated 1,2-bis-(*p*-methoxyphenyl)-1,2-bis-(methylthio)-ethane, which was newly synthesized. The corresponding free phenol and its diacetate had already been synthesized.[5] The dimethyl ether was prepared from hydranisoin and from isohydranisoin as follows:

To obtain hydranisoin and isohydranison anisil was reduced with sodium amalgam in alcoholic solution. The two stereoisomeric products were separated by taking advantage of their different solubilities in ether and recrystallized from alcohol. Hydranisoin, $CH_3OC_6H_4CHOHCHOHC_6H_4OCH_3$, was treated with methyl mercaptan and hydrogen chloride in the same way as described above. Recrystallized from acetic acid, 1,2-bis-(*p*-methoxyphenyl)-1,2-bis-(methylthio)-ethane, $CH_3OC_6H_4CH(SCH_3)CH(SCH_3)C_6H_4OCH_3$, was obtained in colorless prisms melting at 188-190°. Found: S, 19.10%; molecular weight, 335. Calculated for $C_{18}H_{22}O_2S_2$: S, 19.17%; molecular weight, 334. Isohydranisoin, treated similarly, gave a viscous oil and a small amount of the same substance as obtained from hydranisoin. The oily product could not be purified.

Estrogenic Activities. — Since attempted demethylation to free phenols with thiophenol[5] was successful with neither of the hexestrol dimethyl ether analogs described above, the dimethyl ethers were tested on their physiological activities. The minimum doses to produce full estrus in ovariectomized mice by subcutaneous injection in two portions in oil solution were found as follows: 1,2-bis-(*p*-methoxyphenyl)-1-(methylthio)-butane 100 γ, 1,2-bis-(*p*-methoxyphenyl)-1-(methylthio)-2-methoxy ethane 100 γ, 1,2-bis-(*p*-methoxyphenyl)-1,2-bis-(methylthio)-ethylene 10 γ.

The authors thank the Ministry of Education for the Grant in Aid for Fundamental Scientific Research.

Department of Chemistry, Faculty of Science, Tokyo University.

(4) I. Moodie, *J. Chem. Soc.*, **1907**, 543.

(5) *Cf.* G. K. Hughes and E. O. P. Thompson, *Nature*, **164**, 365 (1949).

Studies on the Polarographic Analysis. XI. New Differential Polarography with One Dropping Electrode[1]

By Masayoshi ISHIBASHI and Taitiro FUJINAGA

(Received September 20, 1951)

Introduction

With the progress of polarography, interest in the differential technique has recently been aroused by Heyrovsky[2] and other workers.[3] Their methods depend on the use of two mercury electrodes dropping at the same rate, but this manipulation is very difficult. It was tried to make the drops fall at regular intervals by magnetic force or to make use of a streaming electrode and yet little has been gained in practice.

Delahay deviced to differentiate the electrometric titration curves with regard to time by inserting a condensor in series with a recording galvanometer and Lévèque and Roth have recently applied the method for differentiating the polarographic curves.[4] According to their method, therefore, the differential current can not be measured at any moment while the increase of the applied potential is stopped and also a good reproducibility is scarecely expected because of the use of an electrolytic condensor of a large capacity.

According to the authors' method, in which a rotating current-alternator similar to that of the Kalousek's method is used,[5] two applied potentials different by ΔE are applied by turns and the corresponding electrolytic currents flow through a galvanometer alternatively in reverse directions. Therefore, the differential current with one dropping electrode is easily measured at any applied potential and moreover, when the rotation of the current-alternator is stopped, it comes back to an ordinary polarograph without changing the circuit. These are the differences arising from the use of above mentioned methods.

In conclusion, however, the differential curves obtained by the authors' method show maetrially the differential curve of the ordinary polarogram only when the electrode reaction takes place so rapidly that the diffusion is a rate determinant and also when the reaction is irreversible. On the other hand they take on some different aspects in case of a reversible reaction or of a slow reaction rate.

Instrumentation

As seen from the circuit shown in Fig. 1, when potentiometer drum R and sliding contact C are used, potential difference between A and C, *i. e.*,

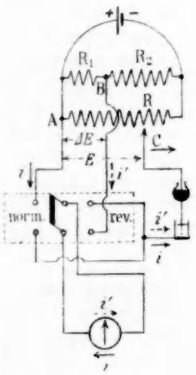


Fig. 1.—The principle of the differential polarograph.

E volt is applied to the cell and by inserting two resistances R_1 and R_2 in parallel with R and connecting B with anode, potential difference between B and C *i. e.*, $E-\Delta E$ volt is applied to the cell. Hence, if a four-poles-double-throw switch and a galvanometer are connected between A, B and the anode as indicated in the figure, a normal polarogram can be obtained when the switch is settled in side "norm.", while a polarogram in which the voltage is consecutively less by ΔE than in the former polarogram is obtained in the contrary direction when the switch is settled in one's turn in side "rev.", so the latter is $E-\Delta E$ *vs.* $-i'$ curve (Curve 2 in Fig. 3). Therefore the galvanometer will indicate $(i-i'/2)=\Delta i$ provided that the changing of the switch connection to side "norm." or "rev." is performed alternatively in regular intervals and moreover, so rapidly as the the galvanometer cannot correspond with the changing of currents because of its

(1) Presented at the annual meeting of the Chemical Society of Japan in 1951.
(2) J. Heyrovsky, *Chem. Listy*, **40**, 222 (1946).
(3) G. Semerano and L. Riccoboni, *Gazz. Chim. ital.*, **72**, 207 (1942).
(4) P. Lévèque and F. Roth, *J. Chim. Physique*, **46**, 480 (1949).
(5) M. Kalousek, Collection *Czech. Chem. Communs.*, **13**, 105 (1948).

inertia. In consequence of this, a differential polarogram, E *vs.* $\Delta i/\Delta E$ curve (Curve 3 in Fig. 3) should be obtained by sliding C continuously along R as in case of an ordinary polarography. To make in practice the connection change of the switch so rapid and at regular intervals, a cylindrical rotating switch shown in Fig. 2 is used and

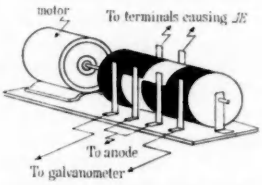


Fig. 2.—The rotating-current-alternator (the differentiator).

the rotation velocity is well adjusted at any interval from 500 to 1,500 r. p. m. Though this rotation velocity of the cylinder is influenced by characteristic properties of galvanometer, 700 to 1,000 r. p. m, is usually adopted (about four times faster than the Kalousek's experiment) and we can carry out the works with much reproducibilities even without using a synchronous motor.

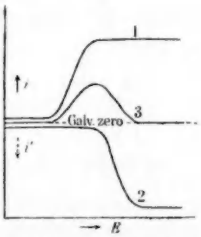


Fig. 3.—Schematic representation of the differential wave: curve 1, normal wave (E~i curve); curve 2, reverse wave ($E-\Delta E$~$-i'$ curve); curve 3, composite differential wave (E~$\Delta i/\Delta E$ curve).

Results Obtained and Discussions

On the Reproducibility.—If ΔE is always fixed and there is not a large fluctuation in the rotation velocity, the results of a good reproducibility can be easily obtained. Fig. 4 indicates the reproducibility of the differential polarograms as for cadmium in chloride solution and as for zinc ammine solution.

On the Relation between the Peak Potential (E_h) of Differential Curve and the Half-Wave Potential ($E_{1/2}$) of Ordinary Curve.—The polarogram of simple metal ion is formulated as below by Heyrovsky and Ilkovic;

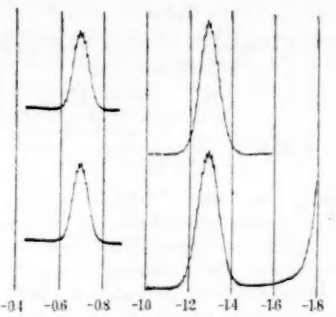


Fig. 4.—Reproducibility of the differential polarograms: left waves, Cd^{++} in KCl; right waves, Zn^{++} in NH_4Cl-NH_4OH.

$$E=E_{1/2}-RT/nF\cdot[\ln i/(i_d-i)] \qquad (1)$$

where E is potential of dropping electrode, $E_{1/2}$ half-wave potential, i electrolytic current corresponding to E, and i_d diffusion current. Here

$$i=i_d/e^{nF(E-E_{1/2})/RT}+1 \qquad (2)$$

Hence, differentiating current i with regard to applied potential E, we get

$$di/dE=-\frac{nF/RT\cdot i_d\cdot e^{nF(E-E_{1/2})/RT}}{\{e^{nF(E-E_{1/2})/RT}+1\}^2} \qquad (3)$$

Suppose d^2i/dE^2 to be zero, then $E=E_{1/2}$. Hence, from the formula (3) we get

$$(di/dE)_{E=E_{1/2}}=-nF\cdot i_d/4RT \qquad (4)$$

So, let E be negative, the differential curve indicates its maximum value at $E_{1/2}$, which is denoted by $nF\cdot i_d/4RT$.

However, the formula (3) is only approximately effective even though ΔE is taken small enough and also the electrode reaction does not always take place fundamentally in accordance with the formala (1), it may be admitted that the peak potential of differential curve is not necessarily in accord with the half-wave potential, But taking $E-\Delta E/2$ instead of E for the potential axis, we can see usually a good accordance of them. (see Table 1). Accordingly, the precise half-wave potential is possible to be measured in qualitative analysis when ΔE is taken small enough.

On the Separability.—In the ordinary polarogram, the half-wave potential is generally on a gentle slope which is apt to give a cause for the overlap of two neighboring waves.

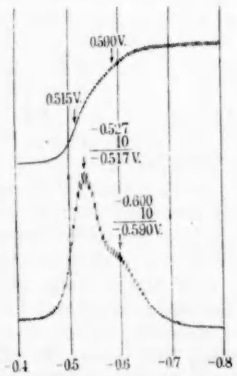
Table 1

Peak Potentials (E_h) and Half-Wave Potentials ($E_{1/2}$)

sample	ΔE, mV.	$Eh = E - \Delta E/2$ (vs. S.C.E.), V.		$E_{1/2}$ (vs. (S.C.E.), V.
Cd^{++} in KCl	20	-0.64_2,	-0.66_5*	-0.64_2
	40	-0.64_4		
	54	-0.64_4		
	54	-0.64_4		
	98	-0.64_6		
	206	-0.65_4		
Zn^{++} in NH_4OH	20	-1.36,	-1.38*	-1.36
	54		-1.38*	
	98		-1.39*	
	206		-1.36*	

* With damped galvanometer

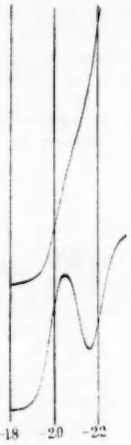
While in the differential polarogram the half-wave potential is denoted by the potential which gives the maximum value of the curve, Moreover, according to this method, the increase in the current often occurs at maximum part owing to the dissolution of the deposited metals and the decrease of it after the maximum takes place by the stoppage of the dissolution phenomena and the minimum between two neighboring maximum is deeply notched. Hence in many cases it is possible to separate the waves whose difference is only 75 millivolts. This availability is more clearly observed particularly in separation at the last increasing domain (Fig. 5 and Fig. 6).



Applied potential, volt

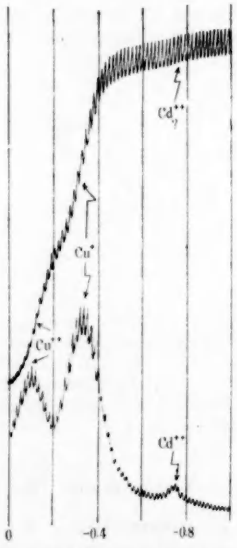
Fig. 5.—Separability of the neighboring waves. Pb^{++} and Tl^{+} in 0.1 *N* KCl—0.1 *N* HNO_3: curve 1, ordinary curve; curve 2, differential curve (ΔE=20 mV.).

On the Elimination of the Components of the Diffusion Current and of the Residual Current.—The effect of the diffusion current is eliminated, so the imperfect detection and determination in the normal polarogram when much formerly discharging matters are present together are quite excluded or considerably lessened and that the sensitivity of the galvanometer is fairly magnified. The result of the experiment is shown in Fig. 7. In the



Applied potential, volt

Fig. 6.—Separability of the neighboring waves. Impurities in crude LiCl: curve 1, ordinary curve; curve 2, differential curve (ΔE=50 mV.).



Applied potential, volt

Fig. 7.—Polarograms of brass dissolved in ammoniacal solution: curve 1, ordinary curve; curve 2, differential curve.

next place as for the residual current, it does not give much interference in the differential polarogram, since it always indicates the constant value. Therefore, the detection of the minute component is well carried out, which is inclined to be obstructed by the comparatively great residual current.

On the Elimination of the Current Vibration.—The current vibration caused by dropping of mercury drops is one of the factors which makes the measurements obscure in the normal polarogram. However, in the authors' differentical method this phenomena should be theoretically excluded; if the potential difference ΔE is enough infinitesimal. At any moment two reverse currents, which are yielded one after another, cancel themselves mutually in the domain of the diffusion current, namely in part excluding the peak in the differencial polarogram. (By the way this elimination does not take place in the Lévèque's method). In practice, however, the vibration is seen all the same in the part of the peak and larger the potential difference is, the larger the vibration becomes. (See also Fig. 7).

Summary

The authors studied on the new differential polarography; its instrumentation and result obtained were particularly reported in the present paper. Discussions for its quantitative analysis and for the electrode reactions and some utilities of current-alternator will be followed.

The authors express their sincere thanks to the Ministry of Education for its financial grant.

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Surface Films of Azo-proteins

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(Received October 2, 1951)

Introduction

Numerous attempts[2] have been made to interprete the observed properties of the protein surface films in terms of the structure and the orientation of the film molecules. Though various hypotheses have been advanced, no definite conclusion has yet been reached. In order to throw some light on this problem, it seemed desirable to study the films of artificially modified proteins and to compare their behavior with that of the original native proteins. For this reason we made a study of the surface films of azo-protein, *i. e.* the protein to the side chains of which known chemical groups have been chemically conjugated through diazonium groups. As a sample of azo-protein, *p*-azo-phenylarsonic acid-conjugated ovalbumin (henceforth referred to as azo-ovalbumin) was chosen. With azo-ovalbumin, experiments were carried out to determine the force-area curves of the films spread on water.

Further, films were transferred, in various ways, onto metal slides and their antigenic activity to the antibody directed specifically against the diazonium groups was tested. In connection with the activity tests, the structure of protein monolayer was discussed. These are described in the present paper.[3]

Experimental

Material.[4]—The samples of azo-ovalbumin with various amounts of diazonium groups were prepared according to the method of Haurowitz[5] from purified hen ovalbumin by treating with different amounts of diazotized *p*-phenylarsonic

(1) Present address: Chemical Laboratory, Faculty of Science, Ochanomizu University, Bunkyo-ku, Tokyo.

(2) H. B. Bull, "Advances in Protein Chemistry," Vol. 3, 1947, p. 95—121.

(3) Following the present study, investigations were made on the relationship between the number of haptenic groups in azo-proteins and the number of adsorbed antibody molecules. These results will be reported elsewhere.

(4) Details on the material will be given by one of the present authors, S. Yamaoka, elsewhere.

(5) F. Haurowitz, *Hoppe-Seylers Z. physiol. Chem.*, **248**, 23 (1936—37).

acid. Each preparation was purified thoroughly until no further color was observed in the supernatant fluid after precipitation by adding hydrochloric acid followed by dialysis. The nitrogen analysis of the compounds was made by the Micro-Kjeldahl method modified by Elek and Sobotka[6] and the arsenic determination by the method of Sandell.[7] Each sample thus obtained was found to be electrophoretically uniform by the Tiselius method.

The number of diazonium groups per one molecule of azo-ovalbumin, x, was estimated by referring to Boyd and Hooker's[8] method of calculation. Then we may write the following equation

$$F_c = F(1-0.373\,R)+3.05\,R \qquad (1)$$

where F_c represents the ratio of the weight of azo-ovalbumin to total nitrogen in it, F, that of ovalbumin to nitrogen which was estimated as 6.62 according to Calvery,[9] and R, that of arsenic to total nitrogen in azo-ovalbumin.

When F_c is obtained from the value of F and R with an azo-ovalbumin, the percentage of arsenic content in azo-ovalbumin, As %, is calculated from the following equation,

$$\text{As}\,\% = 100\times\frac{R}{F_c} \qquad (2)$$

Thus, the value of x is obtained from the following equation

$$\text{As}\,\% = \frac{75\,x}{40000+228\,x}\times 100 \qquad (3)$$

where the molecular weight of ovalbumin is approximately taken as 40,000.

Procedure.—Preliminary experiments showed that although azo-ovalbumins had a tendency to spread on pure water, they appeared to dissolve partly into substrate. Therefore, solution of 0.01 N HCl or, more satisfactorily, a half saturated solution of ammonium sulfate was used as a substrate in a trough of chromium plated brass (60.0×14.6×2.0 cm.) In order to spread azo-ovalbumin monolayer, 0.05% aqueous solution of azo-ovalbumin was given on the surface of substrate solution from a capillary pipette. Force-area curves were obtained by using the Wilhelmy's method based on the principle of tensiometry.[10] The sensitivity of the instrument was of the order ±0.1 dyne per cm. For the measurement in low pressure regions, the sensitivity was raised to 0.01 dyne per cm., using thinner torsion wire (diameter 60 μ). All the experiments were made at a room temperature (about 20°C.). Next, the transfer of azo-ovalbumin monolayers onto metal slides was attempted. However, when acidic substrate water was used, it was frequently unsuccessful to obtain the built-up films of azo-ovalbumin using piston oil of castor oil (pressure, 15 dynes per cm.), because the detachment of the films occurred during the deposition. Thus, azo-ovalbumin was spread on distilled water at pH 5.5 and transferred onto chromium slides covered with the optical gauge of barium stearate multilayers, using castor oil as piston oil. One monolayer of azo-protein film can be transferred either by immersing the slide into the trough before the protein has been spread and withdrawing it through the protein monolayer (up-trip) or by immersing the slide through the protein monolayer previously spread (down-trip) and removing the monolayer from the surface before the slide is withdrawn.

According to Langmuir,[11] the monolayer deposited by down-trip is called an A-layer and that deposited by up-trip, a B-layer. In order to deposit a B-layer directly onto the optical gauge, it was necessary to condition the surface of barium stearate film by thorium nitrate solution (0.001 mol). One double-layer (AB-layer) of azo-ovalbumin can be transferred on stearate layers as easily as native proteins. It was found that a transfer of more than one double-layer on the barium stearate films was not successful, while successive transfer of B-layer was performed without difficulty. However, the increasing number of B-layer made further successive transfer gradually difficult. Possible mode of deposition will be described later.

To measure the deposition ratio, a floating barrier of a circular waxed paper disk was used. The displacement of the disk multiplied by the width of the trough gave the area of monolayer consumed.

The thickness of the built-up films of azo-ovalbumin was measured by using the Blodgett and Langmuir[12] method. The optical thickness (T) of the films was calculated from the following formula:

$$T = 24.4\left(N_1\frac{\cos r_1}{\cos r_2}-N_2\right), \qquad (4)$$

where T is the thickness in Å., N, the number of stearate layers which gives a minimum intensity in the reflected light for the angle of the refraction r_1 before the transfer of the film, N_2 the corresponding number of stearate layers after the film has been transferred, and r_2, the corresponding angle of refraction. The refractive index of protein film was assumed to be 1.495, being the same as that of barium stearate films. It was possible by using polarized sodium light to

(6) A. Elek and H. Sobotka, *J. Am. Chem. Soc.*, **48**, 501 (1926).

(7) E. B. Sandell, *Ind. Eng. Chem., Anal. Ed.*, **14**, 82 (1942).

(8) Boyd and Hooker, *J. Biol. Chem.*, **104**, 329 (1934). In Boyd and Hooker's paper the second term of the righthand side of formula (1) is represented as 3.20 R, but the coefficient should be corrected to 3.05.

(9) Calvery, *J. Biol. Chem.*, **94**, 613 (1931~32).

(10) A. Dognon and M. Abribat, *Compt. rend.*, **208**, 1881 (1939). T. Sasaki, *J. Chem. Soc. Japan.*, **62**, 796 (1941).

(11) I. Langmuir, *Cold Spring Harbor Symp.*, **56**, 171 (1938).

(12) K. B. Blodgett and I. Langmuir, *Phys. Rev.*, **51**, 964 (1937).

measure the thickness of built-up films within the error of approximately ±2Å. The increment of thickness by the adsorption of a antibodies onto azo-ovalbumin films was also measured by the above method.

Results and Discussion

Force-Area Relationships.—(i) High pressure region. Fig. 1 gives the force-area curves for the monolayer of native ovalbumin and azo-ovalbumin on a half-saturated solution of ammonium sulfate. These curves, A, B, C and D, show the relationships between pressure and specific area (m.²/mg.) for a series of azo-ovalbumins of increasing number of diazonium groups. As the number of diazonium groups in azo-ovalbumin molecule increases, the limiting specific area decreases. However, with the area per molecule, instead of specific area of azo-ovalbumin, practically identical force-area relationships were obtained for each sample of azo-ovalbumins irrespective of the number of diazonium groups. In other words, the limiting area (area extrapolated to zero pressure) per one azo-ovalbumin molecule is independent of the number of diazonium groups and is around 7000 Å.², although it tends to increase slightly with the coupling of diazonium groups. This means that diazonium groups introduced contribute not to the area but mainly to the thickness of azo-ovalbumin monolayer. The thickness of monolayer at 15 dynes per cm. can be evaluated from the correspondent specific area and the film density (assumed to be 1.3, the same as density in bulk). The results obtained with the samples of azo-ovalbumin are given in Column VIII of Table 1.

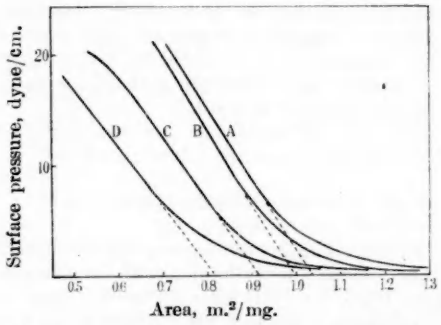

Fig. 1.—Force-area curves for azo-ovalbumin samples: curve A, Native ovalbumin; curves B, C and D, Azo-ovalbumin with 14, 30 and 58 diazonium groups per molecule.

On the other hand, the thickness is also found by the following consideration. Since the area per molecule of each sample is nearly the same at the pressure of 15 dynes per cm. *i. e.*, about 5500 Å.², the thickness (h_A) of their monolayers may be calculated from the following equation.

$$h_A = h_0 \frac{M_A}{M_0}, \qquad (5)$$

h_0 being the thickness of ovalbumin monolayer at the pressure of 15 dynes per cm., calculated from the specific area and density, M_A and M_0, molecular weight of azo-ovalbumin and ovalbumin, respectively, where the latter is taken as 40,000. The thickness values calculated are given in Column IX of Table 1, being in good agreement with that obtained from the specific area and density.

Thus, Eq. (5) is applicable for the estimation of molecular weight of azo-ovalbumins, on the basis of data available from force-area curves. The thickness of a single layer of azo-ovalbumin molecule, when measured directly using the built-up films, gave somewhat higher value than that obtained from force-area relationships (Column X, Table 1). As the deposition ratio of any of the samples of azo-ovalbumin was 0.83, the same as that of native ovalbumin, much higher thickness value should be expected with the film spread on distilled water. This may probably be due to incomplete spreading of azo-ovalbumin which was spread on distilled water so as to form the

Table 1

I	II	III	IV	V	VI	VII	VIII	IX	X
Sample	Number of azo-groups per molecule	Molecular weight	Limiting area per milligram, m.²	Area/mg. at 15 dynes/cm., m.²	Limiting area per molecule, Å.²×10³	Area per molecule at 15 dynes/cm., Å.²×10³	Thickness of monolayer calculated from specific area and density, Å.	Thickness of monolayer from Eq. (5), Å.	Thickness of monolayer observed at 15 dynes/cm., Å.
A	0	40000	1.04	0.81	6.9	5.3	9.5	9.5	9
B	14	43300	1.00	0.78	7.1	5.6	9.9	10.2	11
C	30	47100	0.92	0.69	7.4	5.4	11.1	11.1	12.5
D	58	53800	0.82	0.62	7.3	5.5	13.1	12.7	14

Temperature of measurements: 20~21°C.

built-up films.

It has now been established that the coupled diazonium groups do not contribute to the area of the compressed protein film molecule, but tend to increase the thickness. According to Pauly,[13] and Kapellar-Adler and Boxer[14] the diazonium groups are introduced into the side-chains of polypeptides, such as tyrosine and histidine residues. Accordingly, the present results seem to support the view[15] that under higher pressures the side-chains will be forced out of the surface until they are oriented perpendicular to the surface.

(ii) Low pressure region (below one dyne per cm.). The force-area curves of ovalbumin and azo-ovalbumin with 58 diazonium groups per molecule are shown in Fig. 2. It was found for both samples that a break occurs in force-area curves, denoting a higher order transformation point. A detailed description will be given in future.

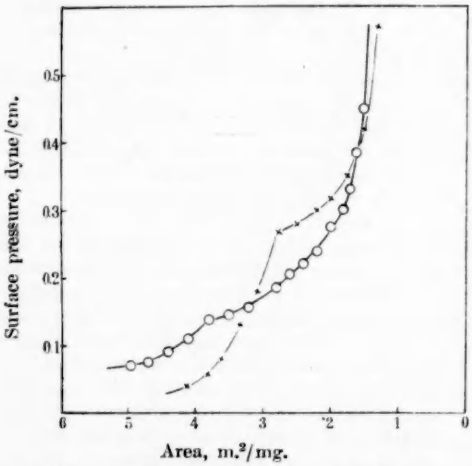

Fig. 2.—Force–area curves of azo-ovalbumin samples: Cross shows native ovalbumin and circle azo-ovalbumin with 41 diazonium groups per molecule. Underlying solution is 0.1 *N* HCl, and temperature, 23°C.

Various Types of Built-up Films of Azo-ovalbumin.—With ovalbumin various types of built-up films have been obtained by Langmuir[11] and coworkers. Langmuir indicated these films with the following symbols: PRA, PRAA, PRAB, PRBBB···, PRBAB, etc., where P stands for a metal plate, R for barium stearate multilayers, A for protein monolayers which is deposited by a down-trip and B for that deposited by an up-trip.

We examined the mode of deposition with azo-ovalbumins and found that it was possible to prepare the following types: PRA, PRABBB ···, PRTB, PRTBB, PRTBAB etc., where RT represents the barium stearate multilayers conditioned by thorium nitrate, the surface of which is hydorphilic. Contrary to ovalbumin, it was difficult to prepare the types of PRB and PRAA. According to our experience, it may be said that the more hydrophobic is the surface of underlying substrate, the easier is the deposition of A-layer, and the more hydrophilic the underlying surface, the easier the deposition of B-layer.

The order of wettability for various type of built-up films gave the following series:

$$\text{PRA} > \text{PRAB} > \text{PRTB}$$

i. e., PRA film is more hydrophilic than PRTB film. Langmuir[11] found, with ovalbumin, that PRA and PRB films differ very little in wettability. This fact has led him to the view that the hydrophobic and hydrophilic portions of protein molecule have no fixed orientations within the film, but can readily overturn by contact with water. In the present experiment, however, B-layer of azo-ovalbumin film was found to have adhered to the PRT film whose surface is highly polar. Then anchoring of hydrophilic groups to the surface of PRT film would occur, leaving the hydrophobic groups on the side exposed to air. This may be the reason why PRA film is more wettable toward water than PRTB film.

Antigenic Activity of Azo-ovalbumin Films.—The reactivity of protein films is evidently associated with the structure and orientation of film molecules. For instance, Langmuir and Schaefer[16] demonstrated that the activity of urease monolayers depends greatly on the type of films deposited. To obtain some insight into this problem, it was desirable to utilize azo–protein–antibody system. For this purpose, various types of the built-up film of azo–ovalbumin containing 29 diazonium groups in one molecule were treated with undiluted antiserum,[17] which contains an

(13) H. Pauly *Z. Physiol. Chem.*, **94**, 284 (1915).
(14) R. Kapellar-Adler and G. Boxer, *Biochem. Z.*, **285**, 55 (1936).
(15) *Cf.* H. Neurath and H. B. Bull, *Chem. Rev.*, **23**, 391 (1938).
(16) I. Langmuir and V. J. Schaefer, *J. Am. Chem. Soc.*, **60**, 1351 (1938).
(17) This serum was obtained from rabbits injected with *p*-phenylarsonic acid-conjugated horse serum and contained appreciable quantity of antibody specifically directed against the diazonium groups.

antibody specifically directed against the *p*-azo-phenylarsonic acid group, according to the following technique. The prepared plates were placed in antiserum during 15~20 hours. The plates were then removed and washed. After drying, the increment of thickness was measured. These results are summarized in Table 2.

Table 2

Type of film	Thickness increment after treatment with antiserum, Å.
PR	—
PRA	225
PRAB	400
$PR(AB)_E$	<20
$PR(AB)_EB$	250
$PR(AB)_EBB$	405
PRTB	90
PRTBA	180
PRTBB	180

The treatments with antiserum were performed at 0°C. The symbol $(AB)_E$ denotes one double-layer of ovalbumin which is ineffective to the adsorption of antibody used in these experiments. All the data were obtained with a pool of antisera in order to eliminate individual variation from serum to serum.

The treatment with antiserum produced a considerable adsorption on respective films, whereas the thickness increment on exposure to normal rabbit serum did not exceed a few Å. Obviously the prepared films demonstrated a clear-cut specificity.

Hereupon it would be of interest to compare the activity of A-layer with that of B-layer. For this purpose it seemed desirable to make a comparison between the activities of PRA and PRB films. However, it was impossible to make the film of type PRB with azo-ovalbumin. Therefore, for the sake of convenience the activity of B-layer on $PR(AB)_E$ film (barium stearate multilayer covered with one double-layer of ovalbumin) was compared with that of A-layer on PR film. The thickness increment with non-specific adsorption on one double-layer of ovalbumin alone ($PR(AB)_E$-film) did not exceed 20 Å. If this non-specific increment is taken from the data in Table 2, it would be found that the activity of a B-layer in $PR(AB)_EB$ is almost equal to that of an A-layer in PRA. Antigenic films of type $PR(AB)_EBB$ and PRAB, also, exhibited equal activity, and the activity of PRAB film was twice as much as that of PRA film. These results show that an A-layer and a B-layer of azo-ovalbumin film differ little in antigenic activity.

In the present experiments, however, the substrate films on which an A-layer or a B-layer was deposited were not the same. In this sense, the experiments using PRTBB and PRTBA as antigenic films would enable us to make the comparison between an A-layer and a B-layer, since then the underlying layer PRTB was common to both films. Thereupon, as shown in Table 2, the thickness increment on both films were almost equal and were twice as thick as that on PRTB film. From these data it may also be concluded that an A-layer and a B-layer have almost equal value in respect to antigenic activity. Similar results have been noticed in the behavior of a single layer of ovalbumin, bovine albumin or metakentrin by Rothen[18] and coworkers.

The results in Table 2 also show that the antigenic activity of azo-ovalbumin layer on PRT film is markedly less than those on PR or $PR(AB)_E$ film. This means that thorium nitrate conditioning of underlying substrate film has an effect of appreciably decreasing on the antigenic activity of azo-ovalbumin layer. This effect may be due to the anchoring of arsonic acid radicals in haptenic groups to highly polar surface of PRT film. The activity of PRTBB and PRTBA films are just twice as much as that of PRTB film which is less than that of PRA film. In other words, the effect of conditioning extends to the top layer of an antigen double-layer. Protein monolayer is of skeleton-like structure as considered by Blodgett[19] so that haptenic groups of top layer also could anchor to the polar surface of conditioned stearate multilayer.

The fact that the activity of two antigen layers is almost twice as much as that of one antigen layer, would require some explanation. Rothen[20] showed with bovine albumin that the amount of specifically adsorbed antibody is directly proportional to the number of underlying antigen layers up to a certain number. He explained this fact by "Long range forces" hypothesis. We also found a similar phenomenon by our experiments with azo-ovalbumin, some results of which were given in the previous paper.[21] In short, owing to the strong combining forces of antigen and antibody, antibody molecules could bind, penetrating into antigen films of skeleton-like structure, with the haptenic groups in the inside of films. Similarities of behavior in A-layer and

(18) A. Rothen: "Advances in Protein Chemistry," Vol. III, p. 123, (1947).
(19) K. B. Blodgett, *J. phys. Chem.*, **41**, 975 (1937).
(20) A. Rothen, *J. Biol. Chem.*, **168**, 75 (1947).
(21) T. Tachibana and K. Fukuda, This Bulletin, **24**, 4 (1951).

B-layer may also be explained along this line.

Summary

(1) Force-area curves were determined of monolayers of native ovalbumin and azo-ovalbumin with various number of diazonium groups. As a results it was found that the coupled diazonium groups do not contribute to the area of compressed protein film molecule, but tends to increase the thickness.

(2) The mode of deposition, when the monolayers of azo-ovalbumins are deposited on metal slides covered with multilayers of barium stearate, was examined. Various types of deposited azo-ovalbumin films thus obtained were tested as to their antigenic activity. The results were discussed in relation to the structure of protein films.

The authors wish to express their gratitude to Professor Jitsusaburo Sameshima (Department of Chemistry) and to Professor Tomio Ogata (Department of Serology) for their kind advice and encouragement throughout this work. The expense of this work has been defrayed from the Grant in Aid for Fundamental Scientific Research from the Ministry of Education, to which the authors' thanks are due.

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Influence of Substituent Groups on Nuclear Reactivity in Formations of Substituted Biphenyls through Reactions of Aromatic Diazo and Cognate Compounds with Aromatic Liquids. II. The Bromine Atom

By Osamu SIMAMURA, Takashi INUKAI and Masaya KURATA

(Received November 24, 1951)

In a previous paper[1] the results of an investigation on the directive influence of the nitro group on the formation of isomeric nitrobiphenyls by the decomposition of N-nitrosoacetanilide or benzenediazo hydroxide in nitrobenzene have been reported. The nitro group has been found to activate all the nuclear positions of nitrobenzene towards substitution by the phenyl radical as compared with the reactivity of the nucleus of benzene itself. The present report is concerned with the results of a similar investigation on the influence of the bromine substituent on the formation of isomeric bromobiphenyls from bromobenzene by the decomposition of N-nitrosoacetanilide or of benzenediazo hydroxide produced by the reaction between benzenediazonium chloride and alkali:

$$C_6H_5Br+C_6H_5N(NO)COCH_3\rightarrow C_6H_5C_6H_4Br+N_2+CH_3COOH,$$

$$C_6H_5Br+C_6H_5N_2Cl+NaOH\rightarrow C_6H_5C_6H_4Br+N_2+NaCl+H_2O.$$

At first we have tried to determine the ratio of *o*-, *m*- and *p*-bromobiphenyls in the product by oxidizing the isomeric mixture formed with chromic trioxide in acetic acid to a mixture of the corresponding bromobenzoic acids and then fractionating the latter. It was shown by preliminary experiments that this method could not give reliable results, because the isomeric bromobiphenyls undergo oxidation with different yields of the corresponding bromobenzoic acids, and, moreover, the yields were hardly reproducible enough to permit the accurate determination. However, it could be demonstrated in this way that all the possible isomerides of bromobiphenyls are produced in these reactions. Thus, the approximate percentages of the *o*-, *m*- and *p*-bromobiphenyls formed in the decomposition of benzenediazo hydroxide in bromobenzene were estimated to be 30.1, 25.5 and 44.4%, respectively, and the corresponding percentages in the decomposition of nitrosoacetanilide in bromobenzene were

(1) O. Simamura, T. Inukai and M. Kanazashi, This Bulletin, **23**, 205 (1950).

22.3, 26.5 and 51.1%, respectively. As the formation of only *o*- and *p*-bromobiphenyls in a similar reaction[2] is recorded in the literature, it is to be noted that the meta isomeride was formed in a considerable amount as in the reaction in nitrobenzene which gave as much as 23% of *m*-nitrobiphenyl along with *o*- and *p*-isomerides.[1] Competitive reactions carried out in a mixture of bromobenzene and benzene showed that bromobenzene reacted with phenyl radicals 1.4 to 2.6 times as fast as benzene.[3]

Snbsequently, infra-red spectrophotometric analysis of the reaction products carried out by Mr. K. Kuratani in the Institute of Science and Technology of this university revealed that the results shown above were too high in the percentage of the para isomeride. In this analysis, absorptions were measured at 9.70, 12.06 and 12.70 microns, each an absorption band characteristic of *o*-, *p*- and *m*-bromobiphenyls, respectively. Table 1 illustrates the accuracy of this analytical method. The results of reinvestigation performed by means of the spectrophotometric determination are summarized in Table 2.

Table 1

Percentages of isomerides in known mixtures

	Taken	Found	Taken	Found
Ortho	60	60	52	50
Meta	11	9	35	34
Para	29	29	13	16

Table 2

Expt. No.*	Reactants		Bromobiphenyls			
		C_6H_5Br	Yield, millimoles	Isomeric ratio *o*	*m*	*p*
1	$C_6H_5N_2OH$ (from 6.5 g. aniline)	200 cc.	19.8	59	26	14
2	$C_6H_5N_2OH$ (from 24 g. aniline)	335 cc.	52.4	53	26	21
3	$C_6H_5N_2OH$ (from 6.5 g. aniline)	217 cc. +C_6H_6 373 cc.	9.18 +Biphenyl 5.96			
4	$C_6H_5N(NO)$-$COCH_3$ 5.0 g	287 cc.	28.8*	60	22	18
5	$C_6H_5N(NO)$-$COCH_3$ 39.5 g.	183 cc. +C_6H_6 155 cc.	22.5 +Biphenyl 8.77			

* See Experimental Part.

The percentage of the ortho isomeride formed was remarkably high, and this is in line with the preponderant formation of *o*-nitrobiphenyl in the reactions of N-nitrosoacetanilide, benzenediazo hydroxide and dibenzoyl peroxide with nitrobenzene, and of *o*-methoxybiphenyl in the decomposition of dibenzoyl peroxide in anisole as shown in Table 3.

Table 3

Reactants		Isomeric ratio of biphenyls formed *o*	*m*	*p*	Reference
$C_6H_5N_2OH$	$C_6H_5NO_2$	46	19	35	(1)
		55	4	41	(4)
$C_6H_5N(NO)Ac$	$C_6H_5NO_2$	58	23	19	(1)
		60	9	31	(4)
$(C_6H_5CO_2)_2$	$C_6H_5NO_2$	72	2	26	(4)
$(C_6H_5CO_2)_2$	$C_6H_5OCH_3$	67	18	15	(5)

In experiment 3, biphenyl and bromobiphenyls were formed in a molar ratio of 1:1.54, and as benzene was used in an amount about twice that of bromobenzene, the ratio of the reactivities of benzene and bromobenzene is 1:3.2. Since it may reasonably be assumed that the isomeric ratio found in experiment 1 obtains in the competitive reaction, the relative reactivities[6] of the *o*-, *m*- and *p*-nuclear positions of bromobenzene are calculated to be 5.7, 2.5 and 2.7, respectively, the reactivity of one position of the unsubstituted benzene nucleus being taken as unity. Similarly, from the data of experiments 4 and 5 the reactivities for the isomeric positions are found to be 4.6, 1.7 and 2.8, respectively.

The common tendency observed in the isomeric ratios of substituted biphenyls formed in the various reactions which include the decomposition of dibenzoyl peroxide and the activating influence of substituent groups, such as the bromine atom (Expts. 3 and 5) and the nitro group,[1] on the reactivity of the benzene nucleus to which these groups are attached towards attack by the phenyl group afford strong supports for a hypothesis that these reactions take place through a mechanism involving free phenyl radicals.[7]

Experimental

Materials.—N-Nitrosoacetanilide was prepared by the method of O. Fischer[8] as in the previous investigation. Benzene, bromobenzene and aniline were purified by repeated distillation.

(2) M. Gomberg and W. E. Bachmann, *J. Am. Chem. Soc.*, **46**, 2339 (1924).

(3) The above results were reported at the third annual meeting of the Chemical Society of Japan, Kyoto, April 1950.

(4) DeLos F. DeTar and H. J. Scheifele, *J. Am. Chem. Soc.*, **73**, 1442 (1951).

(5) T. Suehiro, *J. Chem. Soc. Japan, Pure Chem. Sect.*, **72**, 301 (1951).

(6) See reference 1.

(7) See, *e. g.*, W. A. Waters, "The Chemistry of Free Radicals," Oxford University Press, London, 1948.

(8) O. Fischer, *Ber.*, **9**, 463 (1876).

Reactions with Benzenediazo Hydroxide.—Experiment 1. Aniline (6.5 g.) dissovled in 6 cc. of water and 14 cc. of concentrated hydrochloric acid was diazotized by adding a solution of 5.1 g. of sodium nitrite in 10 cc. of water. The resulting solution was added to 200 cc. of bromobenzene, and to this mixture a solution of 6.7 g. of sodium hydroxide in 20 cc. of water was added dropwise with vigorous stirring at 5-8° in the course of thirty minutes. The stirring was continued for a further four hours. The reaction mixture was separated from the aqueous layer and distilled through a Vigreux column 30 cm. in length to remove bromobenzene. The residue was subjected to vacuum distillation and the distillate treated with a little zinc and hydrochloric acid. The colourless liquid thus obtained was distilled under diminished pressure to yield 4.61 g. of a mixture of bromobiphenyls boiling at 130 to 143° at 8.5 mm. Hg (Found: Br, 34.03%. Calcd. for $C_{12}H_9Br$: Br, 34.28%). Experiment 2 was carried out similarly.

Experiment 3. For a competitive reaction a mixture of 217 cc. (2.02 moles) of bromobenzene and 373 cc. (4.21 moles) of benzene was used instead of 200 cc. of bromobenzene, the amounts of the other reagents being equal to those in the run described above. After the usual treatment benzene and bromobenzene were removed through a fractionating column. The distillate was again fractionated and the residue in the distilling flask was combined with the residue from the initial distillation. The mixture was fractionated under diminished pressure, giving a very small amount of bromobenzene, and then a fraction, from which biphenyl deposited, and another containing bromobiphenyls. The crystallized biphenyl was separated and weighed. The mother liquor was combined with the crude bromobiphenyls, and the mixture was freed from traces of bromobenzene by fractional distillation under diminished pressure and analyzed for bromine in order to estimate the content of bromobiphenyls. Thus, it was found that 2.14 g. of bromobiphenyls and 0.9 g. of biphenyl were produced in this reaction, the yields being 13.2 and 8.5%, respectively, based on the aniline used.

Reactions with N-Nitrosoacetanilide.—Experiment 4. N-Nitrosoacetanilide (5 g.) was added to bromobenzene (286 cc.). The dissolution took place at 16.5 without evolution of heat and the decomposition proceeded gradually. Two such reaction mixtures were combined after three days and worked up as described above. The final product, b. p. 136-145° at 9.5 mm. Hg, weighed 6.72 g. (Found: Br, 34.49%. Calcd. for $C_{12}H_9Br$: Br, 34.28%).

Experiment 5. For a competitive run, 39.5 g. (0.24 mole) of N-nitrosoacetanilide was allowed to decompose in a mixture of 155 cc. of benzene and 183 cc. of bromobenzene (both 1.74 moles), and 5.25 g. of bromobiphenyls and 1.25 g. of biphenyl were obtained.

Summary

The reaction of N-nitrosoacetanilide or benzenediazo hydroxide with bromobenzene gives all three isomerides of bromobiphenyls, the yields decreasing in the order ortho > meta > para. All the nuclear positions of bromobenzene are more reactive than benzene towards attack by the phenyl radicals.

The authors thank Mr. K. Kuratani in the Institute of Science and Technology, Tokyo University for the spectrophotometric analyses and the Ministry of Eduction for a Grant-in-Aid for Scientific Research.

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LETTER

A Note on the Identification of Ortho-, Meta- and Pyro-Phosphates by Means of Paper Chromatography[1]

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(Received December 18, 1951)

It is often desired in the field of biochemical studies to identify easily ortho-, meta-, and pyro-phosphates in minute quantities. A characteristic yellow coloration produced with ammonium molybdate instantly from the ortho-phosphate and only after heating from meta- and pyro-phosphates, will be usefull for detecting them when combined with a development on filter paper. However, it is difficult to distinguish the spots clearly by this method unless the separation is quite complete, as they are all colored similarly. Only for the ortho-phosphate, several methods have been described for its identification, in which the phosphate was detected either on a paper without development by a blue stain formed by adding a drop of molybdate followed by that of benzidine[2] (a

(1) The present paper was read by one of the authors (J. I.) before the 8th Meeting of this institute held on Sept. 29, 1951.

(2) F. Feigl *Z. anal. Chem.*, **61**, 454 (1922); **74**, 386 (1928); **77**, 299 (1929).

spot test), or also on a paper with development by the yellow color of phosphomolybdate or by the blue color formed further from the latter by the action of hydrogen sulfide.[3] Nevertheless, none of these procedures has yet been applied for the identification of meta- and pyro-phosphates.

The present authors have now developed a method of identifying the three types of phosphates accurately on paper chromatograms. About 0.005 cc. of 0.1 *M* aqueous solutions of sodium ortho-, meta- and pyro-phosphates, respectively alone or in their various mixtures, were developed on paper strips (1.8×20 cm. of the Toyo filter paper No. 2) at 25-30°C. with butanol-acetic acid-water (4:1:5 V/V) as a solvent, followed by spraying with a solution of ammonium molybdate in nitric acid[4] and by heating, and then by further spraying with a solution of benzidine in acetic acid.[4] On neutralizing in an atmosphere of ammonia, meta-, pyro- and ortho-phosphates were revealed successively as a blue spot with $R_F = 0$, a purplish red with $R_F = 0.15$, and a blue with $R_F = 0.21$. They were easily distinguished respectively, owing to the difference in color and also to the colorless boundary lines which always appeared among them.

Coexistence of arsenate or nitrate with phosphates was found not to disturb the present identification. Further experiments are now in progress, in which the development with a solvent mixture of modified ratio on the Toyo filter paper No, 50 and the influences of heavy metallic ions upon the chromatograms are respectively examined. Details will be published later elsewhere.

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(3) C. S. Hanes and F. I. Isherwood, *Nature*, **164**, 1107 (1949).

(4) *Cf.* F. Feigl, "Qualitative Analysis by spot Tests," 3rd English edition (translated by R. E. Oesper), New York, 1947, p. 251.

Molecular Structure of Monochloromethyl Chloroformate

By Minoru KASHIMA

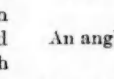
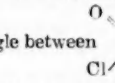
(Received January 21, 1952)

The molecular structure of monochloromethyl chloroformate molecule in the gas phase was determined by electron diffraction studies. The sample was prepared by passing a mixture of methylformate vapour and chlorine through a silica tube exposed to the light from a mercury lamp, and redistilled at 52.5—53° under 100 mm.Hg. The photographs were taken in the usual way with an electron wave lengh of about 0.06 Å. and a camera distance of 8.5 cm.

The interatomic distances and valence angles determined are as follows:

$ClCOOCH_2Cl$.

Cl-C	1.74 ± 0.02 Å.
Cl—C (chloromethyl)	1.74 ± 0.02 Å.
C=O	1.20 ± 0.03 Å.
C—O (carboxyl)	1.40 ± 0.04 Å.
C—O (methoxyl)	1.40 ± 0.04 Å.
∠Cl—C—O	112 ± 4°
∠O=C—O	126 ± 4°
∠C—O—C	110 ± 4°
∠O—C—Cl (chloromethyl)	109.5 ± 3°

An angle between  and  plane

is 0°~90°, and the chlorine atom in the-CH_2Cl group is *trans* to the carboxyl carbon atom.

The carboxyl C—O bond in this molecule is about 0.03 Å. shorter than the normal bond, corresponding about 5% of Pauling double bond character and thus in fair agreement with its non-planar constellation. J. M. O. Gorman *et al.* reported the structures of the methyl formate, methyl acetate and methyl chloroformate molecules by means of electron diffraction.[1] According to their investigation, all these molecules have approximately planar configurations with the ester methyl group *cis* to the carbonyl oxygen atom, and they have shorter carboxyl C—O distance (1.35-1.37 Å.), and longer methoxyl C—O distance (1.46-1.47 Å.), than the normal bond. The contrasting results for these esters are in agreement with the conclusions from dipole-moment studies for aliphatic carboxylic esters[2],[3],[4] but not for methyl carbonate and methyl and ethyl chloroformates, these molecules according to the dipole studies[5],[6] having rather large amplitudes of libration. However, the dipole moment data are not incompatible with a mainly planar structure, either *cis* or *trans*, with considerable libration and considerable resonance, the various, possibly large, resonance moment contributions having been neglected.[5],[6] Accordingly, the carboxylic C—O bond seems to have less double bond character and hindrance to

(1) J. M. O. Gorman, W. Shand, Jr. and V. Schomaker, *J. Am. Chem. Soc.*, **72**, 4222 (1950).

(2) A. Eucken and L. Meyer, *Physik. Z.*, **30**, 397 (1927).

(3) C. T. Zahn, *ibid.*, **33**, 730 (1933).

(4) R. J. B. Marsden and L. E. Sutton, *J. Chem. Soc.* **1936**, 1383.

(5) M. Kubo, Y. Morino and S. Mizushima, *Sci. Papers. Inst. Phys. Chem. Research (Tokyo)*, **32**, 129 (1937).

(6) S. Mizushima and M. Kubo, This Bulletin, **13**, 174 (1938).

libration in methyl chloroformate than in the simple esters, but more than in chloromethyl chloroformate; correspondingly, chlorine in either position probably diminishes the net contribution of the C—O (carboxyl) structures such as I, R—C(O⁻)=O—R′ perhaps by giving rise predominantly to competing structures such as II, Cl⁺=C(O⁻)—O—R′ and III, R—C(=O)—₊O=CH₂Cl⁻ as well as by the destabilizing (inductive) effect of the chloromethyl dipole on structure I. Structure III would also account for the shortening of methoxyl C—O in monochloromethyl chloroformate.

The details of this research will be published elsewhere.

The author wishes to express his sincere appreciation to Dr. S. Yamaguchi, Prof. V. Schomaker and Prof. Y. Morino for their kind advice and discussion, and also to Prof. Y. Kuwata for his interest in carrying out of this research.

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Abnormal Viscosity of Polyvinyl Chloride in Extremely Dilute Solutions

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(Received January 22, 1952)

With a view to determine the intrinsic viscosity of polyvinyl chloride[1] in solutions of some plasticizers, we have measured the viscosity in extremely dilute solutions of unfractionated polyvinyl chloride in dioctyl phthalate as well as in tricresyl phosphate by means of an Ostwald viscosimeter. The solutions were prepared by dispersing the sample in the plasticizer two days at room temperature and by heating four hours at 90°C. The results are shown in Fig. 1,[2] from which, however, the intrinsic viscosity cannot be calculated,

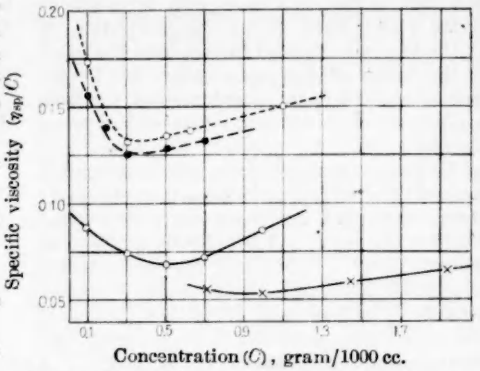

Fig. 1.—Viscosity of polyvinyl chloride in extremely dilute solution: × Sample 1 (average polymerization degree 600), ○ Sample 2 (*ditto* 1500), ● Sample 3 (*ditto* 2900), ——tricresyl phosphate, ... dioctyl phthalate, - - - - cyclohexanone. Temperature: 80°C.

since the relation between the specific viscosity divided by concentration (η_{sp}/C) and the concentration (C) dons not conform with the empirical equation of Huggins.[3] Besides, we have observed a similar abnormal behavior in cyclohexanone at extreme dilution, whereas, at higher concentrations no anomaly has ever been observed in this solvent, as reported in our previous paper.[4] In general, it has been found that the value of η_{sp}/C of polyvinyl chloride in extremely dilute solutions shows a minimum at a certain concentration, and then increases with further dilution. Such behavior has never been found with non-electrolytic high polymers, such as polyvinyl chloride, although similar abnormal phenomena have already been observed with high-molecular electrolyte.[5]

This somewhat unexpected phenomenon may be of particular interest in two respects. First, it is expected that the true intrinsic viscosity of chain polymer is estimated from these experimental results, the value of which is now frequently used for the estimation of the size and shape of chain polymers in solution by the aid of theories. Secondly the concentration of the minimum value of η_{sp}/C changes with the molecular weight of chain polymers and the nature of solvents, which is evident from Fig. 1. Thus, these phenomena together with theoretical considerations suggest that there may exist a certain special interaction between the polymer molecules in such dilute solutions.

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(1) Supplied by the Mitsui Chemical Industrial Company, and purified by precipitating once from a cyclohexanone solution with methanol.

(2) From the fluctuation of measured flow times of solution, the probable error of η_{sp}/C is estimated to be ±1% in the most dilute solution of cyclohexanone, ±3.5% in the most dilute solution of dioctyl phthalate and ±8% in the most dilute solution of tricresyl phosphate.

(3) M. L. Huggins, *J. Am. Chem. Soc.*, **64**, 2716 (1942).

(4) Takeda and Turuta, *J. Phys. Soc. Japan*, **6**, 523 (1951).

(5) Fuoss, *J. Polymer Science*, **3**, 602 (1948).